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13. ABSTRACT (Maximum 200 Words)

The first total syntheses of protesome inhibitors TMC-95A & B have been achieved. Highlights of the synthesis include a venturesome application of the Suzuki biaryl construction, a diastereofacial dihydroxylation reaction taking advantage of the Garner method, and a macrolactamization. A new chemistry to accomplish stereo-specific *cis*-propenamide formation was discovered inspired by goal system. The completion of the total synthesis program paves the way to the development of TMC-95 family compounds as new anti-cancer drug leads.

In addition to the total syntheses of TMC-95A & B, the first total chemical synthesis of diterpene natural product guanacastepene A and its epimers at carbon 5 and carbon 13 position has also been successful accomplished. Thus, the diminished natural source for guanacastepene A has been replaced with this replenishable source. Highlights of the synthesis include a stereo- and regio-selective installation of a α-acetoxyl group via Rubottom-type oxidation, a diastereoselective reduction of ester to hydroxyl group and a subsequent inversion of stereo-chemistry by Mitsunobu reaction, and a chemo-selective oxidation of the primary alcohol in the presence of a secondary alcohol. The synthesis will greatly facilitate the biological study of guanacastepne family compounds, including their potential uses as anticancer agents.

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Total Synthesis of Proteasome Inhibitors TMC-95A-D and Diterpene Guanacastepene A: Towards the Development of New Types of Anti-Cancer Agents

INTRODUCTION

Part I. Total Synthesis of Proteasome Inhibitors TMC-95A-D as Potential Anticancer Agents.

The breakdown of proteins in the body, a process catalyzed by proteolytic enzymes, is a continual process essential to life. The degradation of intramolecular proteins is generally not done by traditional protease but instead carried by a large protein complex called proteasome. The proteasome's central role in protein breakdown accounts for its involvement in an extraordinarily wide range of cellular process-such as cell cycle progress, antigen presentation to immune system, and inflammatory response.[1-3] As the ptoteasome's importance has become more and more apparent over the past few years, it has become not only an attractive object for basic research but a promising target for potential therapeutic development for a range of cellular processes in which the proteasome is involoved, ranging from inflammation to cancer.[4-7]

Recently, increasing attention has been drawn to the development of highly selective proteasome inhibitors as potent chemotherapeutic agents against a variety of human cancers including breast, lung, prostate, leukemia, and colon carcinomas.[8-12] The advantage of this new type of anticancer agents, is that they are potentially less susceptible to multidrug resistance (MDR) and have fewer side effects, which have been the major drawbacks of current clinical drugs, i.e., taxol®. A key factor that could account for fewer side effects, besides the selective inhibitory activity of drugs against protesasome over other cellular enzymes, is tumor cells have been found more susceptible to proteasome-mediated apoptosis than normal cells. [7,9]

TMC-95A (**1a**) and its diastereomers, TMC-95B-D (**1c-d**), are four cyclic peptides recently isolated from the fermentation broth of *Apiospora montagnei* Sacc. TC1093, isolated from a soil sample.[13,14] Biological Studies[13] have shown that TMC-95A inhibited the chymotrypsin-like (ChT-L), trypsin-like (T-L), and peptidylglutamyl-peptide hydrolyzing (PGPH) activities of 20S proteasome (the catalytic core of proteasome[4,15] with IC_{50} values of 5.4 nM, 200 nM and 60 nM respectively. TMC-95-A did not inhibit m-calpain, cathepsin L, and tripsin at 30 μM, suggesting its high selectivity for proteasome. TMC-95A has also showed cytotoxic activities against human cancer cells HCT-116 and HL-60 with IC_{50} values of 4.4 μM and 9.8 μM, respectively. It is thus very promising to develop TMC-95 family compounds into a new type of highly potent anticancer agents. Since the supply from natural sources is limited,[13] chemical synthesis will be the most effective alternative in providing the necessary material for drug development. Following a successful total synthesis, the groundwork would be more promising for establishing systematic SAR profiles of these TMC95 inhibitors. Ideally, simpler structures could be designed, synthesized and screened for similar activity in anticipation of their use as new drug leads. In vitro and in vivo activities again human breast cancer cells will be examined following the completion of the chemical synthesis of TMC-95s. These tests will be done in molecular pharmacology under another grant.
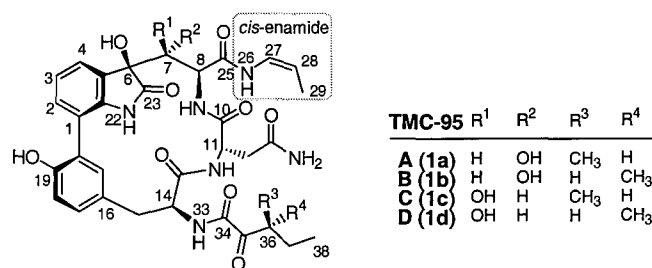
Figure 1. Structures of TMC-95s

TMC-95	R^1	R^2	R^3	R^4
A (1a)	H	OH	CH$_3$	H
B (1b)	H	OH	H	CH$_3$
C (1c)	OH	H	CH$_3$	H
D (1d)	OH	H	H	CH$_3$

Part II. Total Synthesis of Diterpene Guanacastepene A and Its analogs as Potential Anticancer Agents.

Guanacastepene A (**I**, Figure 2) is the parent member of a family of ditepene natural products produced by an unclassified endophytic fungus from the Guanacaste Conservation Area in Costa Rica.[101] Initial interest in guanacastepene A arose from its activity against antibiotic-resistant bacteria. However, subsequent experiments in *E. coli imp* and observed hemolytic activity against human blood cells support nonspecific membrane lysis as its mode of action.[101b,102] Nonetheless, guanacastene A remains a synthetic target of current due to its novel structure and the possibility of exploring activity gunacastepene family in other biological systems.[101c] Particularly, we are interested in investigating the potency of anti-cancer activity of compounds possessing the novel guanacastepene skeleton (guanacastepene A analogs).
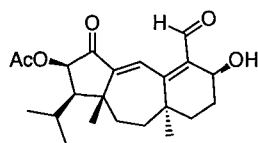

Figure 2. Structure of Guanacastepene A (**I**).

Interestingly, the cultured fungus no longer produces guanacastepene A.[103] At present, chemical synthesis remains as the only access to this compounds. Extensive studies have been reported towards the synthesis of guanacastepene A.[104-107] However, prior to this study, no successful total synthesis of guanacastepene A was reported.

In vitro and in vivo activities again human cancer cells will be examined following the completion of the chemical synthesis of guanacastepene A and its analogs. These tests will be done in molecular pharmacology under another grant.

BODY OF RESEARCH

Part I. Total Synthesis of Proteasome Inhibitors TMC-95A-D as Potential Anticancer Agents.

TMC-95A-D are structurally characterized as novel cyclic peptides containing L-tyrosine, L-asparagine, highly oxidized L-tryptophan, (Z)-1-propenylamine, and 3-methyl-2-oxopentanoic acid moieties. Although the phenyl-indole ring attachment is found in a few natural products such as

chloropeptin,[16] complestatin,[17] diazonamide,[18] and the kistamicins,[19,20] the presence of an oxindole ring in such macrocyclic context is apparently rare. The structural novelty of the TMC95 compounds and the biological issues they raise have stimulated extensive studies directed to their total synthesis.[21-26] We have been playing the leading role in this research area, and have recently completed the synthesis of the macrocyclic core of TMC-95A and B.[21-22]

The apparent synthetic challenges posted by TMC-95s include: a) phenyl-indole ring attachment subunit, b) sensitive α,β-dihydroxyl group susceptible to retro-Aldol cleavage at the up-right of oxindole ring, c) highly constrained macrocycle, and d) unusual, likely reactive and unstable (Z)-1-propenylamide side chain. A key step in our strategy of synthesis is the fashioning of the 17-membered ring by macrolactamization (Scheme 1.) With the ring in place, attentions could then be directed to addition of the unusual, required (Z)-1-propenylamide and 3-methyl-2-oxopentanoate side chains at C-8 and C-14 respectively. The installation of hydroxyl groups at C-6 and C-7 could in principle be conducted prior to, or after, macrocyclization. We further foresaw that a Suzuki reaction,[27] allowing for the joining of 3 + 4, might be employed to reach macrolactamization precursors en route to 2.
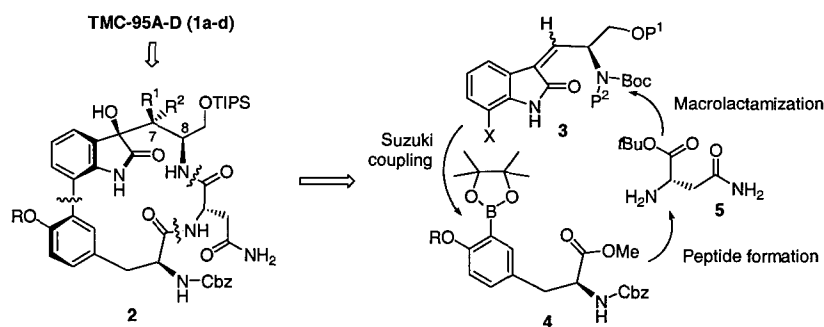

Scheme 1. Synthetic Strategy for TMC-95s

We have demonstrated the efficacy of this synthetic protocol by successful construction of the macrocyclic core **2** (Scheme 1, R = Me, R^1 = H, R^2 = OH).[21] With the encouraging realization of these important milestones during the test of our basic protocol, we continued to pursue our ultimate goal with full confidence. There still remained significant issues to be overcome towards the final total synthesis. First, the installation of unusual, potentially unstable (Z)-1-propenylamide side chain in a stereoselectivity fashion. Secondly, selectivity enhancement en route to macrocyclic core. These include: a) E/Z stereoselectvities during the formation of 3-alkylidene-7-iodooxindole **3**, and b) E/Z stereoselectvities during the Suzuki coupling of aryl borate **4** with iodide **3**, and c) S/R diastereoselectivty in the functionalization of diol subunit at C6-C7 positions. In addition, attachment of the 3-methyl-2-oxopentanoate side chains and the removal of a phenolic methyl ether group can be problematic. Perhaps most worrisome and most inciteful of synthetic innovation, was the cis propenamide linkage, encompassing atoms 26-29 of the multifaceted molecular ensemble of **1**.

Examination of the literature reveals several methods that might in principle be brought to bear on the enamide problem.[28] However, the applicability of this prior art to the culminating phase en route to the active TMC compounds prompted no small concern. Compounds **1** have a rich diversity of functionality, particularly in the extended "pyruvoyl"-like (C34 $\rightarrow$ C38) and dihydroxyindolinone (positions 22, 23, 6, 7, 8 and 28) regions. Anticipation of potential vulnerability in these sectors, not to speak of the cis-enamide itself (C26-C29), prompted us to explore a new modality for reaching such a substructure appropriate for molecules laden with multiple sites of potential instability. We wondered whether a substance of the type **7** might, upon appropriate thermolysis or catalysis, undergo concurrent

ene- and silatropic-like bond reorganizations leading to **8** (Scheme 2). If this hypothesis were to be fruitful, the *cis* character of the enamide would be virtually assured at the kinetic level. Moreover, it seemed at least possible that the intermediate silyl imidate linkage in **8** could be cleaved, with retrieval of general substructure **9**.
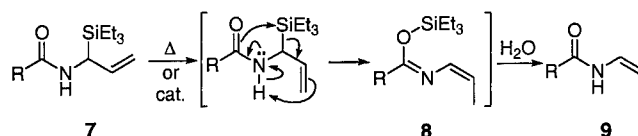
**Scheme 2.** Formation of *cis*-propenamides **9** via rearrangement-hydrolysis of α-silylallyl amides **7** (R = aromatic, alkenyl, and alkyl groups).

In the event, a range of probe substrates **7**, was synthesized by appropriate acylation of the known of amine **6**.[29] As seen in entries a-d, thermolysis of these compounds at ca. 110 °C for the time periods indicated, gave rise to silyl imidates **8a – d** (observed *via* ^{1}H NMR analysis).[30] Aqueous hydrolysis of these compounds afforded enamides **9a – d** (Table 1). The reaction was applicable to substrates **7e** and **7f**, though longer thermolysis times were required for their conversion to **8d** and **8f**. *Most significantly, the thermolysis – hydrolysis sequence proved extendable to the aminoacyl substrate 7g, leading to 8g and thence to 9g*. It was of great interest to determine whether this new method would find application at a very late stage of our projected total synthesis.

Table 1. Thermolysis-hydrolysis of α-silylallyl amides **4**.

| Entry	R	Conditions	yield (%)
a		1) toluene, 110 °C, 10 h; 2) H$_2$O	81
b		1) toluene, 110 °C, 20 h; 2) H$_2$O	73
c		1) toluene, 110 °C, 27 h; 2) H$_2$O	67
d		1) toluene, 110 °C, 3 h; 2) H$_2$O	63
e	Me	1) toluene, 110 °C, 2 d; 2) H$_2$O	73
f		1) toluene, 110 °C, 3 d; 2) H$_2$O	72
g	TIPSO, HN-Boc	1) o-xylene, 135 °C, 4 d; 2) H$_2$O	52

The starting material for our refashioned synthesis was the previously described iodooxindole **10**.[21] Crossed aldol condensation of **10** with the Garner aldehyde **11**[31] followed by β-elimination of the derived mesylate afforded a 1:1.3 mixture of α,β-unsaturated lactams **12(Z)** : **12(E)**.[21,23] Fortunately, the former isomer could be converted to the latter one *via* iodine-mediated isomerization as shown (Scheme 3).[21,23] In a parallel sequence, L-tyrosine (**13**) was converted to **14** in three steps as shown.[32] A

high yielding ortho iodination[21] of **14** led to **15** and thence, following palladium mediated borylation,[33] to **16**. Suzuki type coupling[34] of **16** with **12(E)** afforded compound **17** (75% yield). We hoped that the biaryl domain thus presented, would be serviceable in the context of our projected total synthesis (*vide infra*).
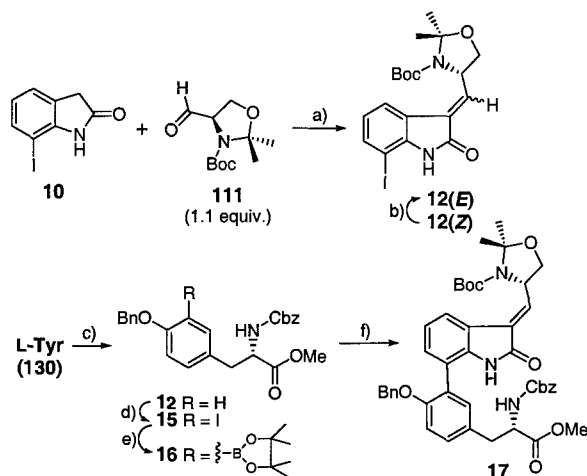
Scheme 3. Synthesis of biaryl compound **17**. key: a) LDA (2.0 equiv.), THF, -78 °C, 1.5 h; TEA, MsCl, CH$_2$Cl$_2$, -70 to -50 °C, 1.5 h; 81% (*E/Z*= 1.3/1); b) I$_2$ (cat.), benzene, 80 °C, 26 h; DMP/PPTS, toluene, 65 °C, 5h; 85% (60% con.); c) 1) MeOH/SOCl$_2$; 2) Cbz-Cl/K$_2$CO$_3$; 3) BnBr,Cs$_2$CO$_3$, acetone, reflux; 88% (3 steps); d) Ag$_2$SO$_4$/I$_2$, MeOH, rt, 1 h; 99%; e) pinacolatodiborane, [PdCl$_2$(dppf)]'CH$_2$Cl$_2$, KOAc, DMSO, 80 °C, 10 h, 91%; f) **12(E)**, [PdCl$_2$(dppf)]'CH$_2$Cl$_2$, K$_2$CO$_3$, DME, 80 °C, 2 h; 75%. Boc = *tert*-butoxycarbonyl, Cbz = benzoxycarbonyl, DMP = 2,2-dimethoxypropane, PPTS = pyridinium *p*-toluenesulfonate, dppf = bis(diphenylphosphino)ferrocene.

Hydrolysis of the methyl ester function of **17** led to the corresponding carboxylic acid, which, following acylation of the basic nitrogen of asparagine derivative **18**, afforded **19**. The hydroxyl groups were introduced at carbons 6 and 7 as shown (Scheme 4). Indeed, the presence of the Garner *N,O*-acetonide served to direct, preferentially, the oxidizing agent to the *Re* face (C6) of **19** (see Scheme 4), thus affording **20** in a 5:1 ratio relative to its 6*R*, 7*S* stereoisomer (not shown).
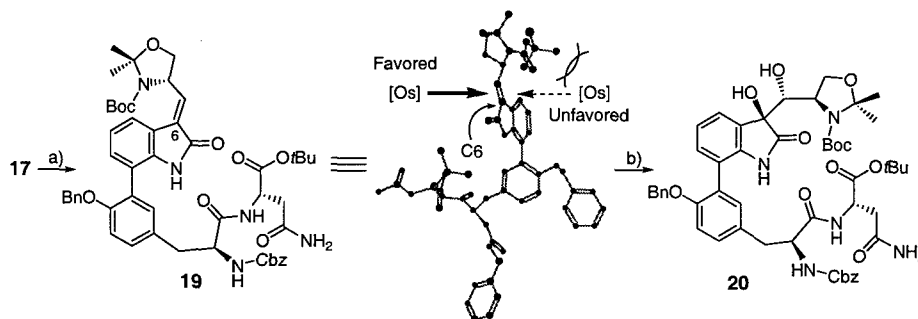
Scheme 4. Synthesis of diol **20**. Key: a) 1) LiOH, THF/H$_2$O, 0 °C, 1.5 h; 2) H-Asn-O*t*Bu (**18**), EDC/HOAT, THF, rt, 2 h; 85% (2 steps); b) OsO$_4$/NMO, (DHQD)$_2$-PHAL, *t*BuOH/H$_2$O, rt, 12 h; 88% (*S/R* = 5/1). Asn = asparagine, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide

The timing and manner in which the various heteroatom-centered functional groups were exposed and protected proved to be critical to the success of the project. We proceeded as follows (see Scheme 5). Deprotection of the N,O-isopropylideneacetal linkage of **20** afforded N-Boc triol **21**. The primary alcohol at position 25 was protected as its TIPS derivative (see **22**). Cleavage of the t-butyl group generated a carboxylic acid at C10, thereby setting the stage for macrolactamization (see compound **23**).[35] In the next step, the benzyl groups protecting the C19 phenol and the nitrogen comprising position 33 were concurrently cleaved by hydrogenolysis. Acylation of the basic nitrogen (position 33) with racemic **24**[36] afforded **25a** and **25b** as a 1:1 mixture. Both compounds were advanced concurrently. The TIPS protecting group was cleaved from the primary alcohol. The four hydroxyl groups (positions 6, 7, 19 and 25) were protected as the tetra-TES derivatives (see **26a** and **26b**). In a key step of the synthesis, reaction of these compounds with Jones Reagent[37] led to specific oxidation at the primary center (position 25) to afford acids **27a,b**. In addition, partial deprotection occurred at the 8C19 ether linkage resulting in the formation of **28a,b**. These four component mixture, following condensation with amine **6** as indicated, led to the amide silyl ethers **29a,b** as well as amide phenols **30a,b** (**29a,b** : **30a,b** ~ 1/1.5).
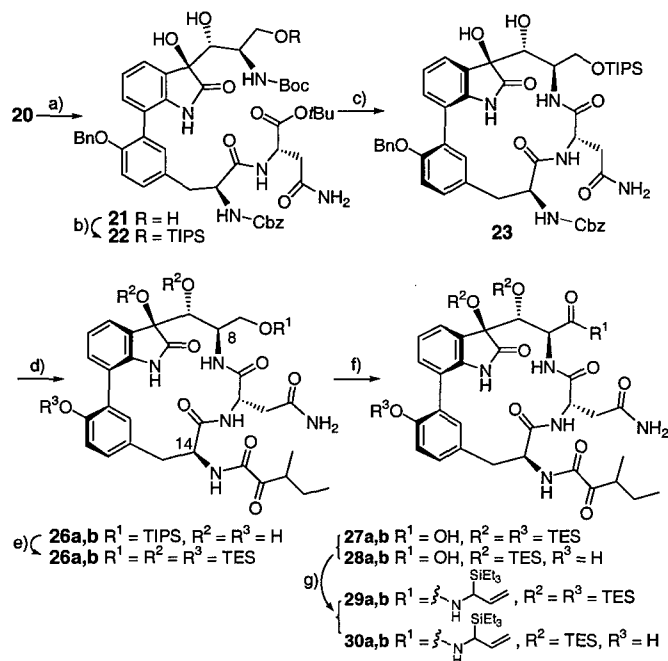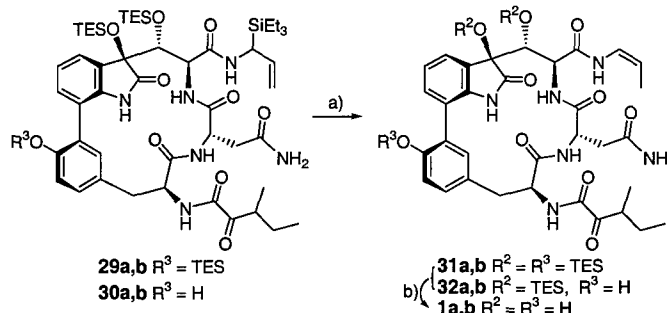

Scheme 5. Synthesis of α-silylallyl amides **29a,b** and **30a,b**. Key: a) PPTS/MeOH, reflux, 2 h; b) TIPS-Cl, imidazole/DMAP, CH_2Cl_2, rt, 5 h; 88% (2 steps); c) 1) TFA/CH_2Cl_2 (4/1), rt, 2h; 2) EDC/HOAT/DIEA, CH_2Cl_2/DMF (2 mM), rt, 24 h; 52% (2 steps); d) 1) Pd/C, H_2, EtOH, rt, 19 h; 2) (±)-3-methyl-2-oxopentanoic acid (**24**), EDC/HOAT, CH_2Cl_2/DMF, rt, 2 h; 85% (2 steps); e) 1) HF/Py; 2) TES-OTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 15 h; 3) $NaHCO_3$; 4) Citric acid, EtOAc/H_2O; 73% (from **25**); f) Jones reagent, acetone, 0 °C, 2 h; g) **6**, EDC/HOAT, CH_2Cl_2/DMF, rt, 13 h; 45% (2steps). DMAP = 4-dimethylaminopyridine, DIEA = N,N-diisopropylethyl amine.

Construction of the (Z)-1-propenylamide was now achieved by thermolytically driven rearrangement of the α-silylallyl amides corresponding to **7** (Scheme 6). The rearrangement of the complex mixture,[38]

in anhydrous *o*-xylene at 140 °C, provided (Z)-1-propenylamides **31a,b** and **32a,b**. The crude mixture of these compounds was globally deprotected with pyridine-buffered HF/pyridine, to afford a mixture of our total synthesis goals – TMC-95A and B (**1a** and **1b**; 1/1). This mixture was separated by HPLC using a reverse phase column[39] to provide the individual compounds **1a** and **1b**. These compounds were characterized by their high field NMR spectra in comparison with those of authentic samples.[39]

29a,b R^3 = TES	**31a,b** R^2 = R^3 = TES
30a,b R^3 = H	**32a,b** R^2 = TES, R^3 = H
	1a,b R^2 = R^3 = H

Scheme 6. Synthesis of TMC-95A and B (**1a,b**). Key: a) 1) *o*-xylene, 140 °C, 3 d; 2) H$_2$O; b) HF/Py, THF/Py; then Me$_3$Si-OMe; 49% (2 steps).

In order to identify pharmacophore and obtained analogs with simpler structure while possessing better biological activity, structure-activity relationship (SAR) study on TMC-95 family is currently being performed. The dramatic difference (20 to 150 times) in biological activities between TMC-95A and B and TMC95C and D (structurally differ only in stereochemistry at C-7 position),[13] as well as the most recent results obtained by Moroder and coworkers, further suggest the necessity of such a study. As shown above, the synthesis presented here is amenable of modifications to furnish various analogs of TMC95s, especially those bearing modifications at C-6, C-7, C-8, C-11, C-14, and C-19 positions (see Figure 3). A series of TMC-95A analogs has been designed and is being synthesized employing the synthetic strategy presented here, and will be evaluated for their biological activities. Results obtained will be carefully analyzed and used in the further rational design and development of TMC-95 family compounds as potent anticancer agents.
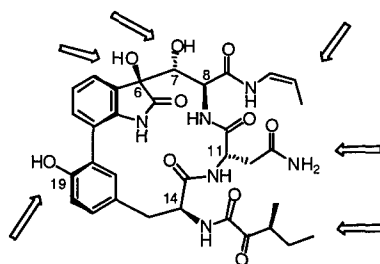

Figure 3. Positions of Interest on TMC-95A (**1a**) for Derivative Synthesis

Part II. Total Synthesis of Diterpene Guanacastepene A and Its analogs as Potential Anticancer Agents.

Earlier work from the our laboratories has demonstrated that the framework of guanacastepene (i.e., **IV**) can be achieved by starting from 2-methyl cyclopentenone **II** via hydroazulene **III**.[107] In order to

synthesize guanacastepene A (**1**), several important factors relating to the densely packed functionality across the northern edge of the molecule, i.e., C5, C15, C14, and C13 positions (Scheme 7), needed to be addressed. Of particular interest were the two stereogenic centers at C5 and C13 positions.
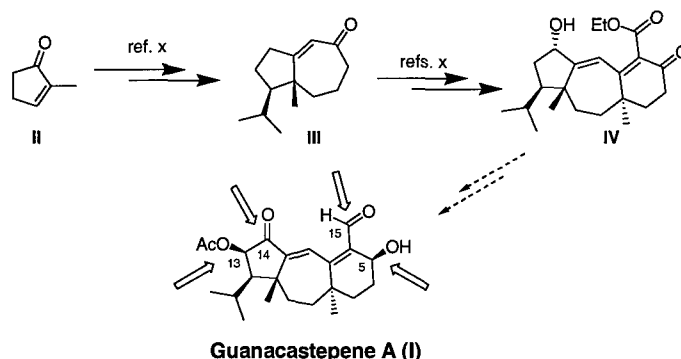
Guanacastepene A (I)

Scheme 7. Synthesis Overview

Through the extensive studies aimed to address the aforementioned issues, the total synthesis of guanacastepene A and its C5 and C13 epimers has been successfully accomplished. The work was in collaboration with Dr. Gregory B. Dudley (NIH postdoctoral fellow) and Dr. Derek S. Tan (Damon Runyon Cancer Research Fund postdoctoral fellow). The optimized synthesis of guanacastepene A (**I**) from compound **IV** is outlined in Scheme 8.
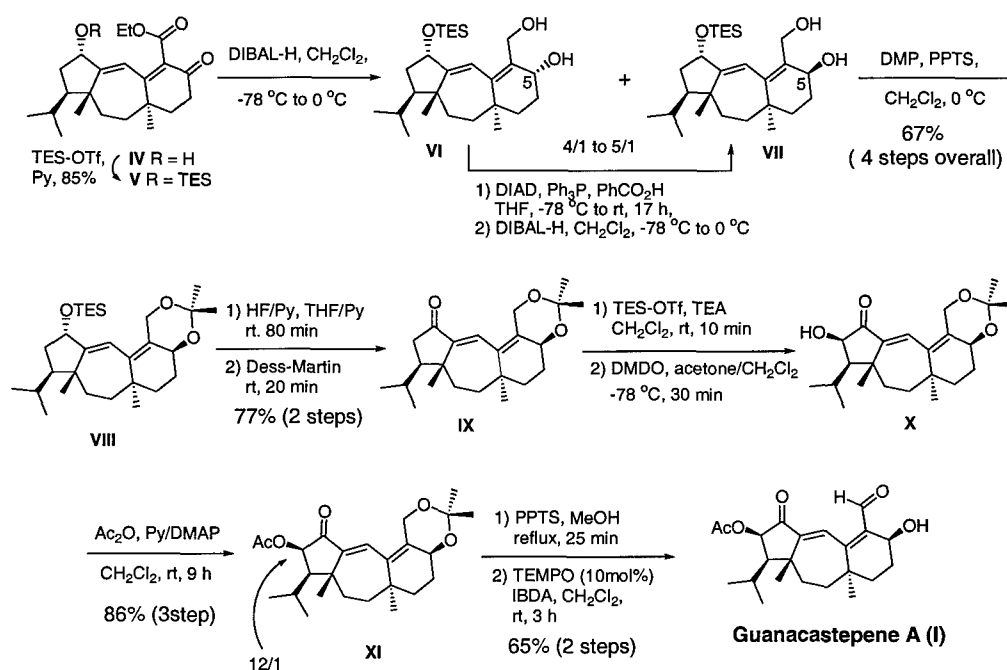
Scheme 8. Synthesis of Guanacastepene A (**I**).

Protection of alcohol **IV** as it TES ether afforded **V** in 85% yield. Treatment of β-keto ester **V** with DIBAL in CH_2Cl_2 provided C5 epimeric diols **VI** and **VII** in ca. 4:1 ratio. Two isomers were separated by silica gel column chromatography, and the α-isomer **VI** was converted to the desired β-isomer **VII**

via Mitsunobu reaction followed by DIBAL reduction of the resulting dibenzoate. Diol **VII** was then protected as acetonide **VIII** in 67% yield over the four steps from compound **V**. Removal of TES protecting group followed by oxidation with Dess-Martin periodinane afforded ketone **IX** in excellent yields. Rubottom-type oxidation,[108] i.e., TES enol ether formation, followed by epoxidaton with DMDO and subsequent aqueous workup, provided α-hydroxy ketone **X**. Aceylation of **X** afforded acetate **XI** with the requisite acetoxy group installed at C13 position in a stereoselective fashion in 86% over three steps. Finally, acetonide protecting group of **XI** was removed by PPTS in Methanol, and the resulting unstable diol was subjected to TEMPO oxidation, which oxidized the primary alcohol to aldehyde and left the secondary alcohol untouched. The desired natural product guanacastepene A (**I**) was obtained in 65% over two steps. The spectral data of the synthetic material (^{1}H and ^{13}C NMR at both 25 °C and -50 °C, IR, MS) are in complete accord with those of natural product.

In the absence of an authentic sample of the natural product for direct comparison, we sought to demonstrate that various epimers of guanacastepene A were indeed distinguishable by NMR spectroscopy. In this light, we synthesized the remaining three diastereomers with respect to C5 and C13 (**Ia, Ib,** and **Ic**) for a comparative analysis. Figure 4 reveals the diagnostic 1H NMR data of the various diastereomers. In short, the epimeric C13 protons exhibit drastically different coupling constants, whereas the epimeric C5 hydroxyls affect the peak shape of the aldehyde signal, presumably through differences in hydrogen bonding.
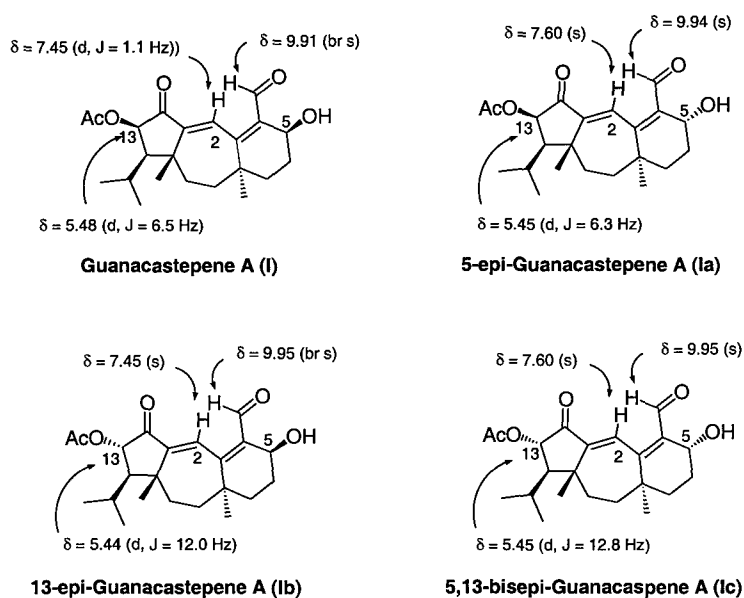

Figure 4. Structures and Key NMR Signals of the Four Epimers

The detailed description of the study can be found in the attached manuscript that has been submitted for publication.[109]

KEY RESEARCH ACCOMPLISHMENT

Part I. Total Synthesis of Proteasome Inhibitors TMC-95A-D as Potential Anticancer Agents

- Successful accomplishment of the first total chemical syntheses of natural occurring proteasome inhibitors TMC-95A and TMC-95B. Highlights of the synthesis include a venturesome application of the Suzuki biaryl construction, a diastereofacial dihydroxylation reaction, a macrolactamization, and a cis-enamide formation.
- Provision of an alternative supply source for TMC-95A & TMC-95B;
- Discovery of a novel organic transformation: Stereo-specific synthesis of cis-propenamides via thermo-rearrangement of α-silylallyl amides.
- Facilitation of the design and synthesis of TMC-95 family proteasome inhibitors as potential anticancer agents.

Part II. Total Synthesis of Diterpene Guanacastepene A and Its analogs as Potential Anticancer Agents

- Successful accomplishment of first total chemical synthesis of diterpene natural product guanacastepene A. Highlights of the synthesis include a stereo- and regio-selective installation of a α-acetoxyl group via Rubottom-type oxidation, a diastereoselective reduction of ester to hydroxyl group and a subsequent inversion of stereo-chemistry by Mitsunobu reaction, and a chemo-selective oxidation of the primary alcohol in the presence of a secondary alcohol.
- Provision of a reliable supply source in replacement of the diminished natural source for guanacastepene A;
- Pavement the way of quick access to other members of gunacastepene family natural products. 5-*epi*-, 13-*epi*-, and 5,13-*bisepi*-guanacastepene A have been synthesized utilizing the strategy/technique developed.
- Facilitation of the design and synthesis of guanacastepne family compounds as potential anticancer agents as well as drugs for other disorders.

REPORTABLE OUTCOMES

1. The following publications/manuscripts are based on the research work supported by this award:

   - Lin, S. and Danishefsky, S. J. "The Total Synthesis of Proteasome Inhibitors TMC-95A & B: Discovery of A New Method to Generate *cis*-Propeny Amides." *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 512-515; *Angew. Chem.* **2002**, *114*, 530-533.
   - Lin, S. and Dudley, G. B.; Tan, D. S.; Danishefsky, S. J. "A Stereoselective Route to Guanacastepene A via a Surprising Epoxidation." *Angew. Chem. Int. Ed. Engl.* **2002**, submitted.
   - Lin, S., Yang, Z. Q.; Danishefsky, S. J. "Total Synthesis and Biological Study of Proteasome Inhibitors TMC-95A & B and Their Analogs." Manuscript under preparation.

2. The following provisional patent application is filed partially based on the research work supported by this award:

   - Danishefsky, S. J. and Lin, S. "Cyclic Peptides, Analogues and Uses Thereof." United State Provisional Patent Application, filed January 07, 2002.

3. The following employment/research offers made to P.I. (Dr. S. Lin) were partially based on the experience/training supported by this award:

- *Senior Research Scientist*, Merck & Co, Inc, Rahway, New Jersey (November, 2001);
- *Senior Scientist*, Schering-Plough Research Institute, Kenilworth, New Jersey (October, 2001);
- *Scientist*, R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey (November, 2001);
- *Senior Scientist*, Boehringer-Ingelheim Pharmaceuticals, Ridgefield, Connecticut (November 2001).

4. The following news/public attentions were based on the research work supported by this award:

- Borman, S. "Enamide Transformation. Novel Construction Procedure is Key to Proteasome Inhibitor Total Syntheses." *News of the Week* Section, *Chemical & Engineering News*, page 14-14, March 04, 2002.

CONCLUSIONS

In summary, the first total syntheses of TMC-95A & B have been achieved. The program featured a sequential assembly of oxindole **10**, Garner's aldehyde (**11**), aryl boronate **16**, asparagine derivative **18**, 3-methyl-2-oxopentanoic acid (**21**), and α-silylallyl amine (**3**). Highlights of the synthesis include a venturesome application of the Suzuki biaryl construction (**912E**) + **16** → **17**), a diastereofacial dihydroxylation reaction taking advantage of the Garner method (**19** → **20**), and a macrolactamization (formation of **23**). We also note new chemistry to accomplish stereo-specific *cis*-propenamide formation (**29** & **30** → **31** & **32**) was inspired by goal system **1**. The successful application to the delicate case at hand serves to build confidence in its likely generality. The completion of the total synthesis program paves the way to the development of TMC-95 family compounds as new anti-cancer drug leads.

In addition to the total syntheses of TMC-95A & B, the first total chemical synthesis of diterpene natural product guanacastepene A and its epimers at carbon 5 and carbon 13 position has also been successful accomplished. Thus, the diminished natural source for guanacastepene A has been replaced with this replenishable source. Highlights of the synthesis include a stereo- and regio-selective installation of a α-acetoxyl group via Rubottom-type oxidation, a diastereoselective reduction of ester to hydroxyl group and a subsequent inversion of stereo-chemistry by Mitsunobu reaction, and a chemo-selective oxidation of the primary alcohol in the presence of a secondary alcohol. The synthesis will greatly facilitate the biological study of guanacastepne family compounds, including their potential uses as anticancer agents.

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39) HPLC conditions are as follows. Column: YMC-pack ODS-AM, 150 X 10 mm; eluant: 25% MeCN in water; flow rate: 2.5 mL/min; T_{R1a} = 37.3 min, T_{R1b} = 34.3 min. The synthetic

materials were identical to natural TMC-95A & B (Rf, NMR, EIMS, and HPLC). We thank Dr. Jun Kohno, Tanabe Seiyaku Co., Japan, for generously providing us with the HPLC conditions and the samples of TMC-95A & B for comparison .

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APPENDICES

(See Attachments)

1) Lin, S. and Danishefsky, S. J. "The Total Synthesis of Proteasome Inhibitors TMC-95A & B: Discovery of A New Method to Generate *cis*-Propeny Amides." *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 512-515; *Angew. Chem.* **2002**, *114*, 530-533.

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Silatropic and ene-like bond reorganizations (see scheme, left) were the key steps in the first total synthesis of the title compounds, which only differ in stereochemistry at the remote C36 stereocenter. Other key steps include a Suzuki biaryl construction, a diastereofacial dihydroxylation reaction, and a macrolactamization.
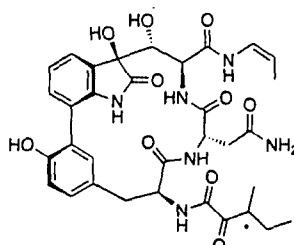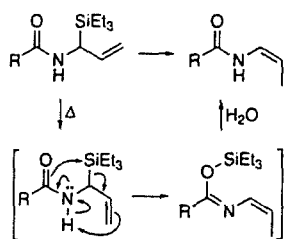
TMC-95A/TMC-95B

Angew. Chem. **2002**, *114*, 530–533

Keywords: biaryls · inhibitors · macrolactamization · rearrangement · total synthesis

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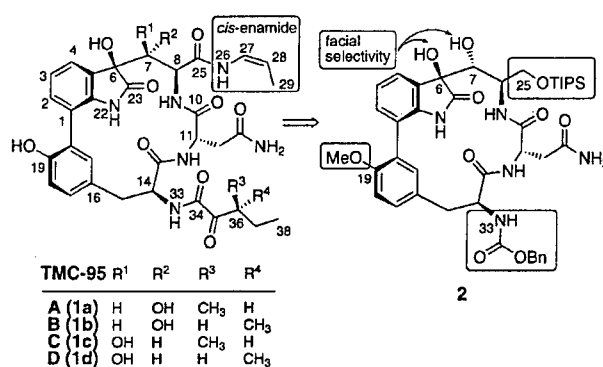* Author to whom correspondence should be addressed

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The Total Synthesis of Proteasome Inhibitors TMC-95A and TMC-95B: Discovery of a New Method To Generate cis-Propenyl Amides**

Songnian Lin and Samuel J. Danishefsky*

The ubiquitin proteasome pathway is critical for accomplishing proteolysis in both the cytosol and nucleus of all eukaryotic cells.[1] Understanding of the physiological roles of a particular proteasome has been aided by studying the effects of cell-permeable inhibitors. Moreover, specific proteasome inhibitors are emerging as possible drug candidates.[1c] Accordingly, the discovery of the cyclic peptides TMC-95A – D (1a–d; see Scheme 1), first isolated as fermentation products of *Apoispora montagnei* SACC TC 1093 derived from soil samples,[2] was of great interest.[3, 4] The A and B isomers of 1, which differ in stereochemistry at the remote, configuratively labile C36 center, inhibit proteasomal functions of the 20S proteasome with IC_{50} values down to low nanomolar levels.[2a]

Accordingly, a total synthesis program, which targets the more active TMC-95A and B isomers, was launched in our laboratory. We hoped to gain access to both compounds since after a successful total synthesis the groundwork would be more promising for establishing systematic SAR profiles of these TMC-95 inhibitors. Ideally, simpler structures could be designed, synthesized, and screened for similar activity in anticipation of their use as new drug leads.

Recently, we described an approach to accomplish stage I in our program, that is, the total synthesis of the TMC-95 inhibitors.[4a] The most advanced structure reported in our previous disclosure was compound 2 (Scheme 1). The attainment of this subgoal, promising as it was for our proposed program, still left unaddressed several key issues for total syntheses of 1a and 1b. Thus, provision would be necessary to allow exposure of a free phenolic hydroxy group at C19 and a homopyruvoyl group at N33 (see structure 2). Moreover, the oxidation level at C25 must be upgraded from an alcohol, as found in 2, to the carboxy state required to reach 1 (see below). This was no trivial matter, given the free hydroxy groups at C6 and C7 in our model target, 2. We also note that in the synthesis of 2 the facial sense of cis dihydroxylation at

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Scheme 1. Structures of TMC-95A – D (1a–d) and 2. TIPS = triisopropylsilyl, Bn = benzyl.

TMC-95	R^1	R^2	R^3	R^4
A (1a)	H	OH	CH_3	H
B (1b)	H	OH	H	CH_3
C (1c)	OH	H	CH_3	H
D (1d)	OH	H	H	CH_3

C6 and C7 was virtually stereorandom.[4a] Perhaps most problematic was the cis-propenyl amide linkage that encompasses atoms N26 – C29 of the multifaceted molecular ensemble of 1. It is with this latter issue that we commence our report.

Examination of the literature reveals several methods that might, in principle, be relevant to the enamide problem.[5] However, the applicability of these methods to the synthesis of the active TMC compounds is not certain. Compounds 1 have a rich diversity of functionality, particularly in the extended "pyruvoyl"-like (C34 – C38) and dihydroxyindolinone (positions 22, 23, and 6 – 8) regions. Anticipation of potential vulnerability in these sectors, not to speak of the cis-enamide itself (N26 – C29), prompted us to explore a new modality for reaching such a substructure appropriate for molecules with multiple sites of potential instability. We wondered whether a substance of the type 3 might, upon heating or catalysis, undergo concurrent ene- and silatropic-like bond reorganizations that would lead to 4 (Scheme 2). If this hypothesis were to be fruitful, the cis character of the enamide would be virtually assured at the kinetic level. Moreover, it seemed at least possible that the intermediate silyl imidate linkage in 4 could be cleaved, with retrieval of general substructure 5.
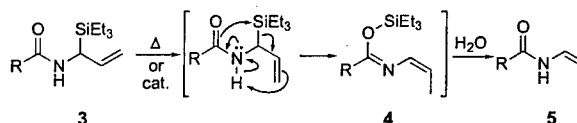
Scheme 2. Formation of cis-propenyl amides 5 by rearrangement – hydrolysis of α-silylallyl amides 3 (R = aryl, alkenyl, and alkyl groups).

In the event, a range of probe substrates 3 was synthesized by acylation of the known of amine 6 (Table 1).[6] As seen in entries 1 – 3, heating of these compounds at about 110 °C for the time periods indicated gave rise to silyl imidates 4a – c (observed by ^{1}H NMR analysis).[7] Aqueous hydrolysis of these compounds afforded enamides 5a – c. The reaction was also applicable to substrate 3d (entry 4), though a longer heating time was required for its conversion to 4d. Most significantly, the rearrangement – hydrolysis sequence proved extendable to the aminoacyl substrate 3e (entry 5) to afford

Table 1. Rearrangement – hydrolysis of α-silylallyl amides 3.[a]

Entry	R =	Conditions	Yield [%]
1	a	a) toluene, 110°C, 10 h; b) H$_2$O	81
2	b	a) toluene, 110°C, 20 h; b) H$_2$O	73
3	c	a) toluene, 110°C, 27 h; b) H$_2$O	67
4	d	a) toluene, 110°C, 3 d; b) H$_2$O	72
5	e	a) o-xylene, 135°C, 4 d; b) H$_2$O	52

[a] TES = triethylsilyl, Boc = tert-butoxycarbonyl, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOAT = 1-hydroxy-7-azabenzotriazole.

4e and thence 5e. It was of great interest to determine whether this new method would find application at a very late stage of our projected total synthesis.

The starting material for our refashioned synthesis was the previously described iodooxindole 7 (Scheme 3).[4a] Crossed-aldol condensation of 7 with the Garner aldehyde 8,[8] followed by β-elimination of the derived mesylate, afforded
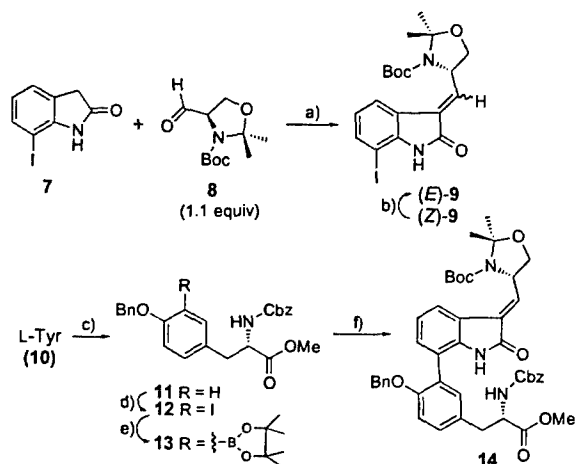
Scheme 3. Synthesis of biaryl compound 14. a) LDA (2.0 equiv), THF, −78°C, 1.5 h; NEt₃, MsCl, CH₂Cl₂, −70 → −50°C, 1.5 h; 81% (E/Z = 1.3:1); b) I₂ (cat.), benzene, 80°C, 26 h; DMP/PPTS, toluene, 65°C, 5 h; 85% (60% conversion); c) 1) MeOH/SOCl₂; 2) CbzCl/K₂CO₃; 3) BnBr, Cs₂CO₃, acetone, reflux; 88% (three steps); d) Ag₂SO₄/I₂, MeOH, RT, 1 h; 99%; e) pinacolatodiborane, [PdCl₂(dppf)]·CH₂Cl₂, KOAc, DMSO, 80°C, 10 h, 91%; f) (E)-9, [PdCl₂(dppf)]·CH₂Cl₂, K₂CO₃, DME, 80°C, 2 h; 75%. Cbz = benzyloxycarbonyl, LDA = lithium diisopropylamide, DMP = 2,2-dimethoxypropane, PPTS = pyridinium p-toluenesulfonate, dppf = bis(diphenylphosphanyl)ferrocene.

a 1:1.3 mixture of α,β-unsaturated lactams (Z)-9 and (E)-9.[4a,e] Fortunately, the former isomer could be converted to the latter one through iodine-mediated isomerization.[4a,e] In a parallel sequence, L-tyrosine (10) was converted to 11 in three steps (Scheme 3).[9] A high-yielding ortho iodination[4a] of 11 led to 12 and thence, after palladium-mediated borylation,[10] to 13. Suzuki-type coupling[11] of 13 with (E)-9 afforded compound 14 (75% yield). We hoped that the biaryl domain thus presented would be serviceable in the context of our projected total synthesis (see below).

Hydrolysis of the methyl ester function of 14 led to the corresponding carboxylic acid, which, after acylation of the basic nitrogen atom of the asparagine derivative H-Asn-OtBu (15), afforded 16. The hydroxy groups were introduced at C6 and C7, as shown in Scheme 4. Indeed, the presence of the Garner N,O-acetonide served to direct, preferentially, the oxidizing agent to the Re face (C6) of 16 to afford 17 in a 5:1 ratio relative to its 6R,7S stereoisomer (not shown).
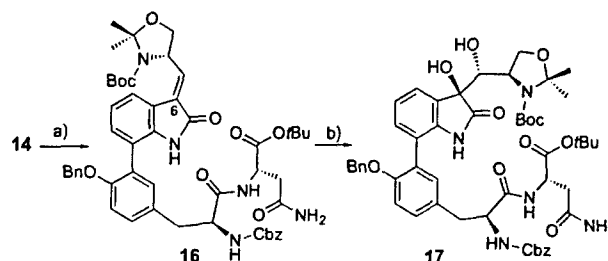
Scheme 4. Synthesis of diol 17. a) 1) LiOH, THF/H₂O, 0°C, 1.5 h; 2) H-Asn-OtBu (15), EDC/HOAT, THF, RT, 2 h; 85% (two steps); b) OsO₄/NMO, (DHQD)₂-PHAL, tBuOH/H₂O, RT, 12 h; 88% (dr = 5:1). Asn = asparagine, NMO = 4-methylmorpholine N-oxide, (DHQD)₂-PHAL = 1,4-bis(9-O-dihydroquinidine)phthalazine.

The timing and manner in which the various heteroatom-centered functional groups were exposed and protected proved to be critical to the success of the project. We proceeded as follows (see Scheme 5): Deprotection of the N,O-acetonide linkage of 17 afforded N-Boc triol 18. The primary alcohol at position 25 was protected as its TIPS derivative (19). Cleavage of the tert-butyl group generated a carboxylic acid at C10 which set the stage for macrolactamization (see compound 20).[12] In the next step, the benzyl groups protecting the C19 phenol and N33 were concurrently cleaved by hydrogenolysis. Acylation of the latter basic nitrogen atom with racemic 21[13] afforded 22a and 22b as a 1:1 mixture. Both compounds were advanced concurrently. The TIPS protecting group was cleaved from the primary alcohol. The four hydroxy groups (positions 6, 7, 19, and 25) were protected as the TES derivatives (see 23a and 23b). In a key step of the synthesis, reaction of these compounds with Jones reagent[14] led to specific oxidation at the primary center (position 25) to afford acids 24a and 24b. In addition, partial deprotection occurred at the C19 ether linkage which resulted in the formation of 25a and 25b. This four-component mixture led, after condensation with amine 6 as indicated, to the amide silyl ethers 26a and 26b as well as amide phenols 27a and 27b (26a,b:27a,b ≈ 1:1.5).
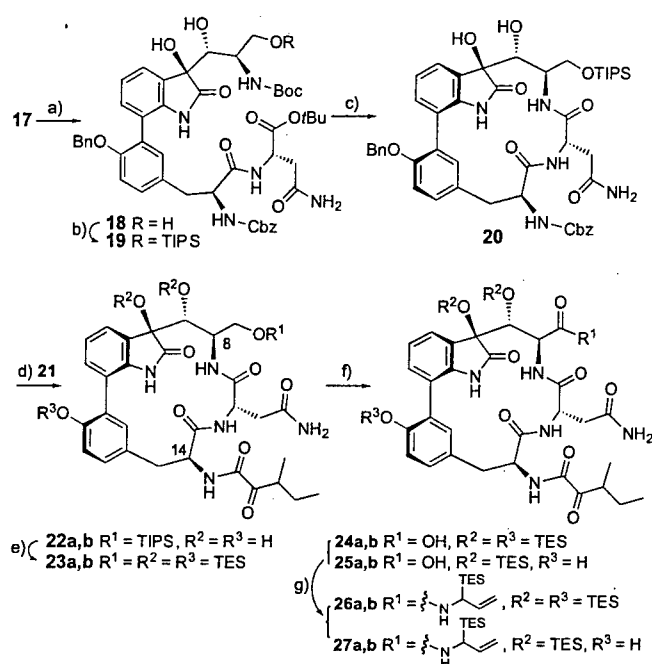
Scheme 5. Synthesis of α-silylallyl amides **26a,b** and **27a,b**. a) PPTS/MeOH, reflux, 2 h; b) TIPSCl, imidazole/DMAP, CH₂Cl₂, RT, 5 h; 88% (two steps); c) 1) TFA/CH₂Cl₂ (4:1), RT, 2 h; 2) EDC/HOAT/DIEA, CH₂Cl₂/DMF(2 mM), RT, 24 h; 52% (two steps); d) 1) Pd/C, H₂, EtOH, RT, 19 h; 2) (±)-3-methyl-2-oxopentanoic acid (**21**), EDC/HOAT, CH₂Cl₂/DMF, RT, 2 h; 85% (2 steps); e) 1) HF/Py; 2) TESOTf, 2,6-lutidine, CH₂Cl₂, 0°C→RT, 15 h; 3) NaHCO₃; 4) citric acid, EtOAc/H₂O; 73% (from **22**); f) Jones reagent, acetone, 0°C, 2 h; g) **6**, EDC/HOAT, CH₂Cl₂/DMF, RT, 13 h; 45% (two steps). DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, DIEA = N,N-diisopropylethyl amine.

Construction of the (Z)-1-propenylamide was now achieved by thermally driven rearrangement of the α-silylallyl amides corresponding to **3** (Scheme 6). The rearrangement of
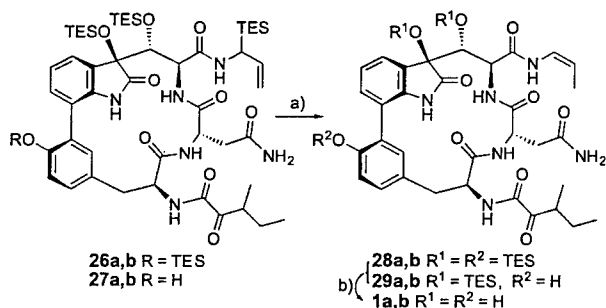
Scheme 6. Synthesis of TMC-95A (**1a**) and TMC-95B (**1b**). a) 1) o-xylene, 140°C, 3 d; 2) H₂O; b) HF/Py, THF/Py; then Me₃SiOMe; 49% (two steps).

the complex mixture[15] in anhydrous o-xylene at 140°C provided (Z)-1-propenylamides **28a,b** and **29a,b**. The crude mixture of these compounds was globally deprotected with pyridine-buffered HF/pyridine to afford a mixture of our total synthesis goals—TMC-95A and TMC-95B (**1a** and **1b**; 1:1). This mixture was separated by RP-HPLC[16] to provide the individual compounds **1a** and **1b**. These were characterized
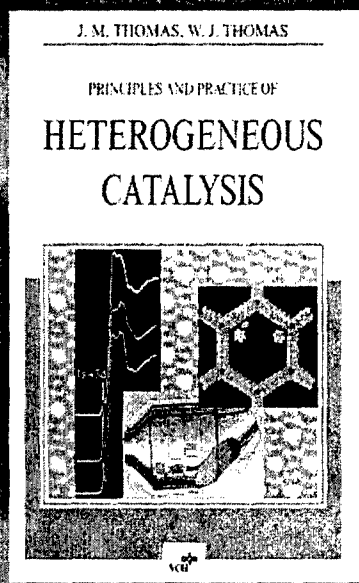
by their high-field NMR spectra in comparison with those of authentic samples.[16]

In summary, the total syntheses of TMC-95A and TMC-95B have been achieved. The program featured a sequential assembly of oxindole **7**, Garner's aldehyde (**8**), aryl boronate **13**, asparagine derivative **15**, 3-methyl-2-oxopentanoic acid (**21**), and α-silylallyl amine **6**. Highlights of the synthesis include a Suzuki biaryl construction ((E)-**9** + **13**→**14**), a diastereofacial dihydroxylation reaction that took advantage of the Garner method (**16**→**17**), and a macrolactamization (formation of **20**). We also note that new chemistry to accomplish stereospecific cis-propenyl amide formation (**26/27**→**28/29**) was inspired by goal system **1**. The application to the delicate case at hand serves to build confidence in its generality.

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[1] a) A. Ciechanover, A. L. Schwartz, *Proc. Natl. Acad. Sci. USA* **1998**, 95, 2727–2730; b) M. Bochtler, L. Ditzel, M. Groll, C. Hartmann, R. Huber, *Annu. Rev. Biophys. Biomol. Struct.* **1999**, 28, 295–317; c) A. F. Kisselev, A. L. Goldberg, *Chem. Biol.* **2001**, 8, 739–758.

[2] a) Y. Koguchi, J. Kohno, M. Nishio, K. Takahashi, T. Okuda, T. Ohnuki, S. Komatsubara, *J. Antibiot.* **2000**, 53, 105–109; b) J. Kohno, Y. Koguchi, M. Nishio, K. Nakao, M. Kuroda, R. Shimizu, T. Ohnuki, S. Komatsubara, *J. Org. Chem.* **2000**, 65, 990–995.

[3] M. Groll, Y. Koguchi, R. Huber, J. Kohno, *J. Mol. Biol.* **2001**, 311, 543–548.

[4] a) S. Lin, S. J. Danishefsky, *Angew. Chem.* **2001**, 113, 2020–2024; *Angew. Chem. Int. Ed.* **2001**, 40, 1967–1970. For synthetic efforts directed to the TMC-95 metabolites from other laboratories, see: b) M. Inoue, H. Furyama, H. Sakazaki, M. Hirama, *Org. Lett.* **2001**, 3, 2863–2865; c) D. Ma, Q. Wu,. *Tetrahedron Lett.* **2001**, 42, 5279–5281; d) B. K. Albrecht, R. M. Williams, *Tetrahedron Lett.* **2001**, 42, 2755–2757; e) D. Ma, Q. Wu, *Tetrahedron Lett.* **2000**, 41, 9089–9093.

[5] a) J. K. Stille, Y. Becker, *J. Org. Chem.* **1980**, 45, 2139–2145; b) P. Ribereau, M. Delamare, S. Celanire, G. Queguiner, *Tetrahedron Lett.* **2001**, 42, 3571–73; c) K. Kuramochi, H. Watanabe, T. Kitahara, *Synlett* **2000**, 3, 397–399; d) R. Chen, J. A. Porco, Jr., *Org. Lett.* **2000**, 2, 1333–1336.

[6] Amine **6** (S.-F. Chen, E. Ho, P. S. Mariano, *Tetrahedron* **1988**, 44, 7013–7026) was synthesized by using an improved procedure from allyl alcohol through a one-pot TES ether formation, retro-Brook rearrangement, and mesylation, followed by a displacement of mesylate with ammonia. Acylation in entries 1–4 (Table 1) was accomplished by coupling of **6** with the acid chlorides, while in entry 5 an EDC-mediated coupling with protected amino acid with amine **6** was involved. Details will be forthcoming in a full disclosure.

[7] The formation of silyl imidates **4** was clearly observed by ¹H NMR spectroscopy when these reactions were carried out in deuterated solvents.

[8] a) P. Garner, J. M. Park, *J. Org. Chem.* **1987**, 52, 2361–2364; b) A. McKillop, R. K. Taylor, R. J. Watson, N. Lewis, *Synthesis* **1994**, 31–33. Garner's aldehyde was employed since it was found that an N,O-acetonide was crucial for the stereoselective installation of diol functionality at C6–C7 position (see ref. [4a]).

[9] To address the issues raised by problematic deprotection of the phenolic methyl group at a later stage (that is, compound **2**, Scheme 1), a benzyl group, instead of a methyl group, was used for the protection of the phenol (see ref. [4a]).

[10] a) T. Ishiyama, M. Murata, N. Miyaura, *J. Org. Chem.* **1995**, 60, 7508–7510; b) A. M. Elder, D. H. Rich, *Org. Lett.* **1999**, 1, 1443–1446.

[11] For a review of Suzuki coupling reactions, see: N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, 95, 2457–2483.

[12] The atropisomer shown for **20** follows from the C6 stereochemistry (see ref. [2b]).

[13] 3-Methyl-2-oxopentanoic acid (**21**) was obtained from its commercially available sodium salt. There would be no purpose in coupling with enantiomerically defined acid **21** because the C36 stereocenter epimerizes rapidly throughout the series.

[14] a) K. Bowden, I. M. Heibron, E. R. H. Jones, B. C. L. Weedon, *J. Chem. Soc.* **1946**, 39; b) R. A. Pilli, M. M. Victor, *Tetrahedron Lett.* **1998**, *39*, 4421–4424.

[15] It was not feasible or necessary to separate at this stage. Rather the eight-component mixture was advanced as shown, and the separation was achieved at the stage of the two-component mixture **1a/1b**.

[16] HPLC conditions are as follows. Column: YMC-pack ODS-AM, 150 × 10 mm; eluant: 25 % MeCN in water; flow rate: 2.5 mL min^{-1}; t_R(**1a**) = 37.3 min, t_R(**1b**) = 34.3 min. The synthetic materials were identical to natural TMC-95A and TMC-95B (TLC, NMR, EIMS, and HPLC). We thank Dr. Jun Kohno, Tanabe Seiyaku Co., Japan, for generously providing us with the HPLC conditions and the samples of TMC-95A and B for comparison.

A Stereoselective Route to Guanacastepene A via a

Surprising Epoxidation**

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In the preceding paper we reported on the preparation

of compound **3**,[1] which bears much of the functionality

required, in principle, to reach guanacastepene A.[2,3] In

order to accomplish our goal,[4] it would be necessary to

reproduce, through chemical synthesis, the densely packed

and varied functionality between carbons 13 and 5. Of

particular interest were the two stereogenic centers yet to

be fashioned. The enhancement of the oxidation level at

carbon 13, ultimately in the form of a β-disposed acetoxy

substituent, had to be properly orchestrated with oxidation at carbon 14 and overall two-electron reductions at carbons 5 and 15.

[Scheme 1. Synthesis Overview]

guanacastepene A (1)
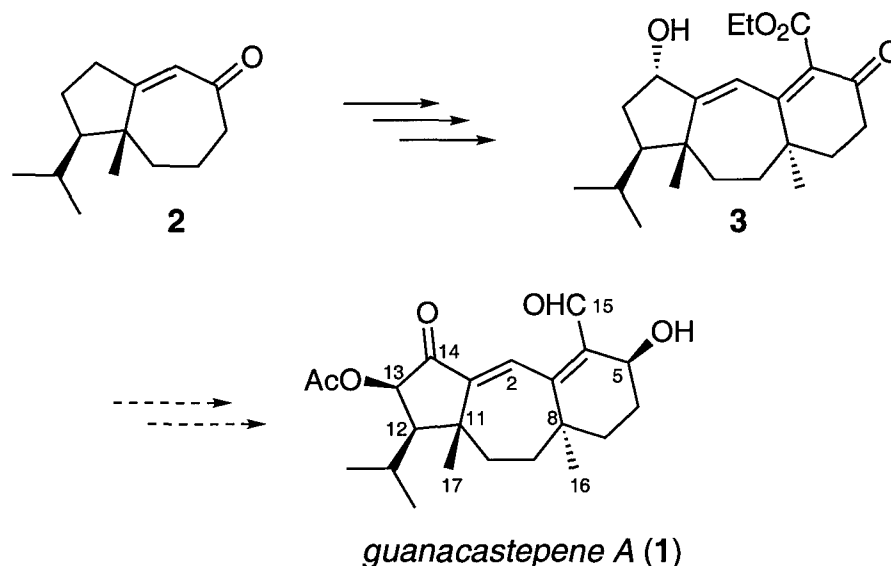
Introduction of the stereogenic center at carbon 5 is described in Scheme 2. The sequence was initiated by protection of the free hydroxyl of 3 as its triethylsilyl ether to give 4. Reduction of the latter with DIBAL-H in CH2Cl2 provided an 80:20 mixture of C4-diastereomers.[5,6] Use of various alternative reducing agents, including LiAlH4, Li(t-BuO)3AlH,[7] 9-BBN,[8] and NaBH4•CeCl3,[9] led to less favorable diastereomeric ratios. The diastereomers (5 and 6) can be separated by careful purification on silica gel.[10] The α-epimer (5, major) was subjected to a Mitsunobu[11] inversion sequence via an intermediate dibenzoate. Protection of diol 6 as its acetonide, cleavage of the

2

silyl ether, and Dess–Martin oxidation[12] (in the presence of pyridine)[13] provided ketone **7**. In initial experiments prior to optimization of the Mitsunobu inversion, diol **5** was advanced to ketone **8** by a sequence analogous to the transformation of **6** to **7**.

[Scheme 2. Preparation of the keto-acetonide]
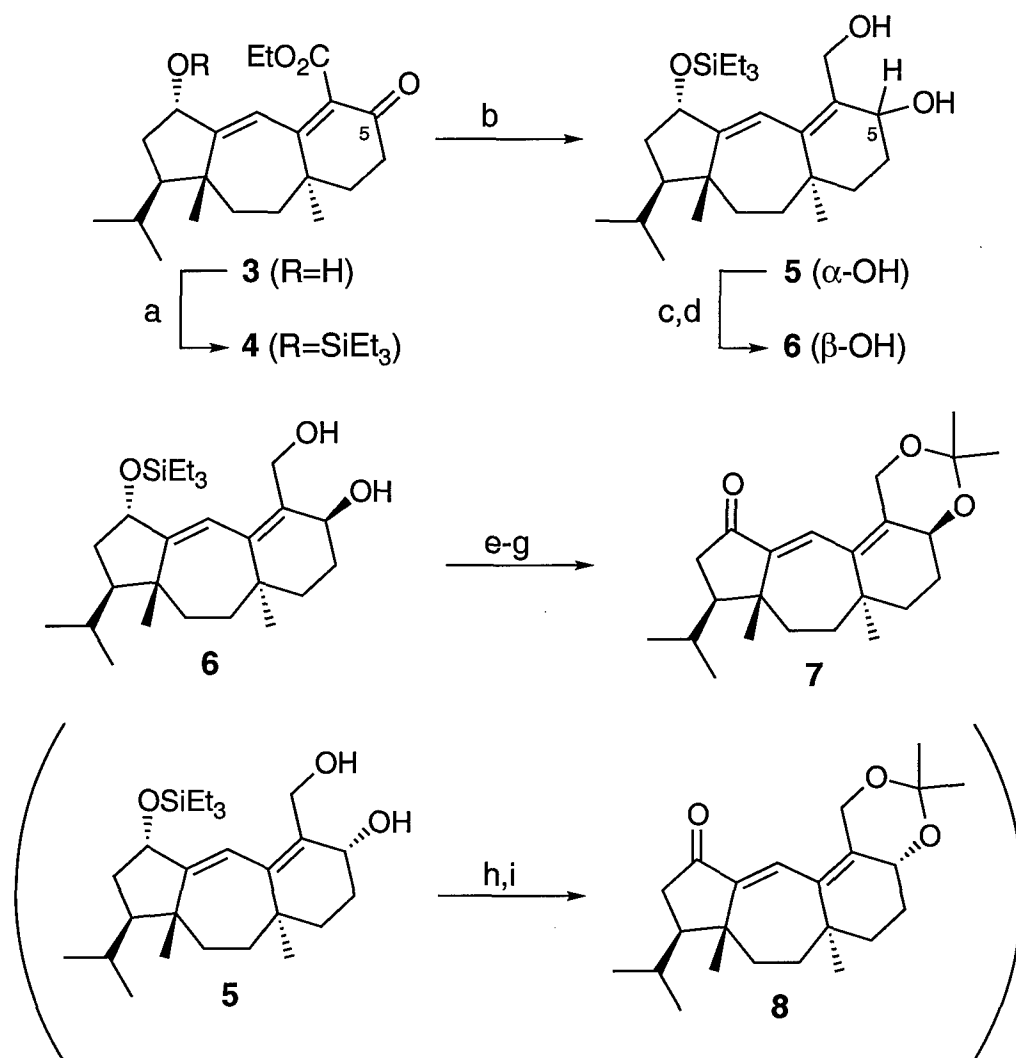
Key: (a) TESOTf, pyridine, CH2Cl2, 0 °C, 80–85%; (b) DIBAL, CH2Cl2, –78 → 0 °C (**5a/5b** = 80/20); (c) Ph3P, BzOH, DIAD, THF, –78 °C → room temperature; (d) DIBAL, CH2Cl2, –78 → 0

°C; (e) dimethoxypropane, PPTS, CH2Cl2, 0 °C, 67% from **4**; (f) TBAF, THF, 0 °C, 91-98%; (g) Dess-Martin periodinane, pyridine, CH2Cl2, 90%; (h) dimethoxypropane, PPTS, CH2Cl2, 0 °C, 86%; (i) HF•pyridine, pyridine, THF, then Dess-Martin periodinane, CH2Cl2, 77-85%.

For the stereoselective installation of the C13-acetoxy substituent, we envisioned creation of C13-C14 enol derivative, for the moment unspecified, shown as **9** (Scheme 3). The conversion **9 → 11** is intended to show, in formal terms, oxidation at carbon 13. At this stage, the nature of the step to introduce the acetyl function is also not specified. For instance, there might be a sequence consisting of hydroxylation at carbon 13 followed by acetylation. Alternatively, the delivery of the "acetyl" and "hydroxenium" components of the overall C13-acetoxy group might be coordinated. At this stage it was expected that oxidants attacking C13 would do so from the α-face, i.e. *anti* to the β-disposed C12-isopropyl and C11-methyl groups. This presumption led to a more specific proposal.

Enol acetylation of a C14-ketone would give **9** (R = Ac). Following the bias discussed above, epoxidation might well lead to **10**. Two stereochemically diverging pathways suggest themselves in advancing from epoxide type **10** to an acetoxy ketone. In path *a*, we emphasize the vulnerability

4

of the C14-O bond of the epoxide. This bond cleavage could lead to loss of MeCO$^+$ to adventitious external nucleophiles and formation of a C13-hydroxyl function requiring acetylation. Alternatively, but still within the confines of path *a*, the acylium function may be transferred internally to the emerging C13-hydroxyl group. It will be noted that in either case path *a* leads to an acetoxy ketone (*cf* **12**) in which the stereochemistry at carbon 13 is the same as that of the epoxide. By contrast, in path *b* one contemplates transfer of an intact acetoxy function from C12 → C13. Here of course the stereochemistry of C13 will reflect the inversion event in the acetoxy migration (*cf* **10** → **11**).[14]

Scheme 3. Acetoxy installation overview

9

[O]

+Ac$^+$ (−R$^+$)

path b

11

inversion at C13

10

path *a* (+Ac$^+$)

12

retention at C13
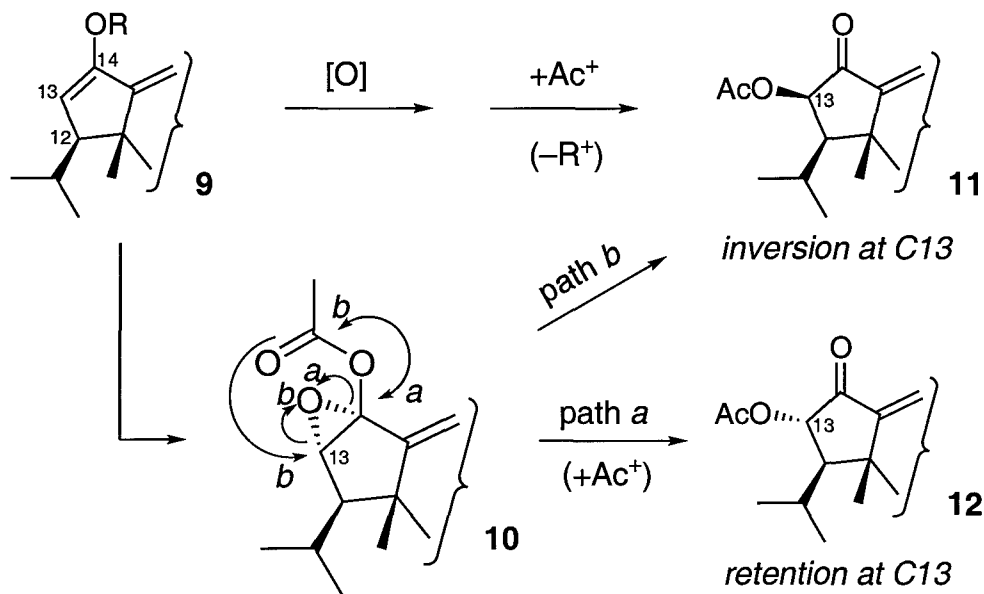
Since the keto-acetonide that first came into our possession was **8** (Scheme 2), we proceeded to test our strategy in this series. Before relating our results it is well to describe our method of analysis even before reaching guanacastepene A. In ketone **8**, two vicinal coupling constants (13.2 Hz and 7.6 Hz) relate the geminal protons at C13 with the α-proton at C12. Noting that $J_{H13\alpha\text{-}H12\alpha} \approx 7$ Hz in the natural product,[21] we could assign the 13.2 Hz coupling to H13β-H12α, while J = 7.6 Hz is assigned to H13α-H12α. This assignment was substantiated by observation of the indicated NOE correlations (Scheme 4). The hope was to assign the stereochemistry of the C13-acetoxylated product by measuring $J_{H13\text{-}H12}$ in the context discussed above.

In the event, ketone **8** was converted to enol acetate **13** (Scheme 4).[15] Epoxidation was conducted with dimethyldioxirane, leading to the formation of a single observable epoxide. The stereochemistry of the oxido linkage (*cf* **13a** or **13b**) was not known at this stage. We were surprised to find that when the compound was subjected to path *a*-favoring (acidic) conditions, followed by acetylation as shown, compound **14** bearing the guanacastepene A-like vicinal H13-H12 coupling constant was obtained. By contrast, recourse to pyrolysis of the epoxy-

6

acetate, hoping to favor the path *b* (inversion) conditions, two compounds were ultimately obtained.[16] One was the acetoxy ketone corresponding to the previously encountered **14**. The other was a new acetoxy ketone, **15**, with a coupling constant suggestive of a *trans* relationship of the C13 and C14 protons. *Assuming that the assignments at C13 in **14** and **15** are correct, these data can only be rationalized by the surprising conclusion that epoxidation of the enol acetate of **8** had occurred from the β-face (see **13b**).*

Scheme 4. Acetoxy installation
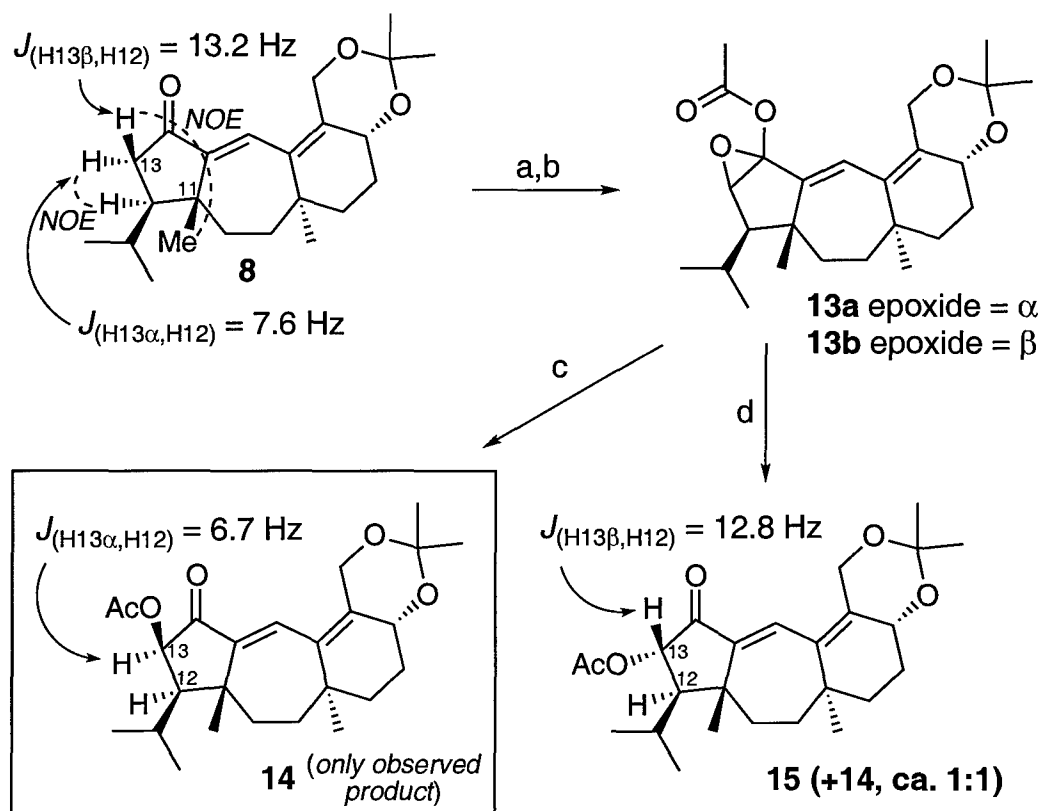
Key: (a) Et3N, DMAP, AcCl, Ac2O, 100 °C, 90%; (b) DMDO/acetone, CH2Cl2, −78 → 0 °C; (c) *p*-TsOH, MeNO2, then Ac2O, pyridine, DMAP, ca. 60% from **8**; (d) 150 °C (neat), then Ac2O, pyridine, DMAP, ca. 65% from **8** (**14/15**, ca. 1/1).

From this newly gained insight, it was opportune to direct efforts toward the synthesis of guanacastepene A itself. For this we commenced with keto-acetonide **7** (Scheme 5). Since we were seeking retention at C13, it was advantageous to employ a Rubottom-type protocol.[17] Conversion of **7** to its silyl enol ether was followed by exposure of the latter to the action of DMDO, thereby providing hydroxy-ketone **16** (ca. 94:6 dr, major isomer shown). Acetylation gave **17**. As before, the diagnostic coupling constants demonstrated that acetoxylation had occurred *syn* to the isopropyl and methyl substituents. Since the Rubottom protocol can be safely assumed to occur by retention,[18] we must conclude that epoxidation had again occurred from the β-face. The assignment of the stereochemistry of **17** was confirmed by its ultimate transformation to guanacastepene A as shown in Scheme 6.

Scheme 5. Rubottom oxidation

$J_{(H13\beta,H12)} = 13.3$ Hz

$J_{(H13\alpha,H12)} = 7.6$ Hz

$J_{(H13\alpha,H12)} = 6.4$ Hz
(R = OAc)

7

16 (R=H)

17 (R=OAc)
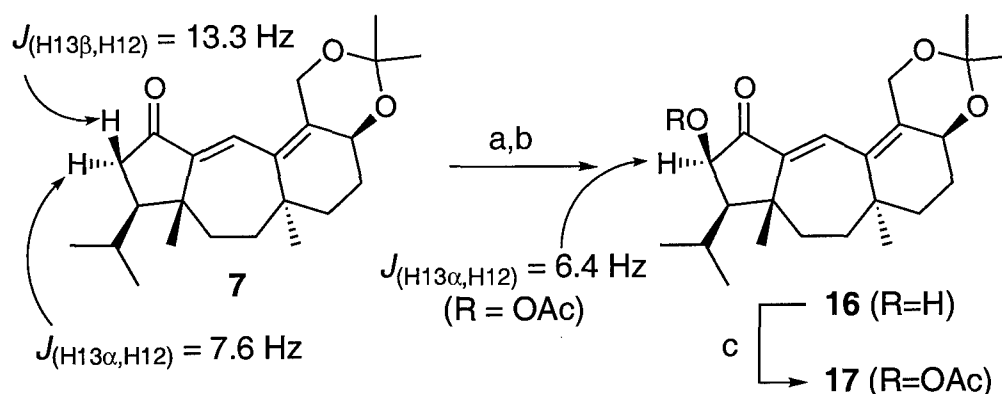
Key: (a) TESOTf, Et3N, CH2Cl2; (b) DMDO/acetone, CH2Cl2, -78 °C, then Me2S, 82-90% overall; (c) Ac2O, pyridine, DMAP, CH2Cl2, 96%.

Hydrolysis of the acetonide of **17** provided an unstable diol, which was purified quickly by chromatography on silica gel and used immediately. TEMPO-catalyzed oxidation of the primary alcohol[19] to the aldehyde left the secondary alcohol undisturbed. The spectral data recorded from this product (1H NMR at 25 °C and at -50 °C, 13C NMR at 25 °C and at -50 °C, IR, and mass spectra) were in complete accord with those of guanacastepene A.[20,21]

[Scheme 6. Hydroxy ketone to guanacastepene A]

17
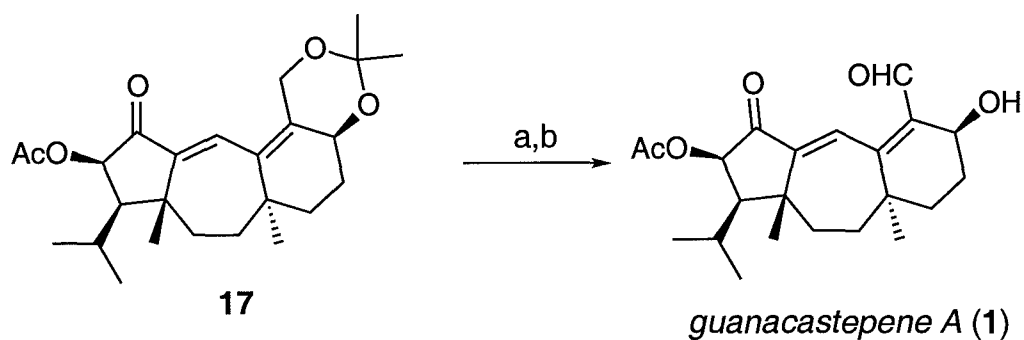
guanacastepene A (**1**)

Key: (a) PPTS, MeOH, 70 °C; (b) PhI(OAc)2, TEMPO, CH2Cl2, 59–65% overall.

We were at this point confident that the total synthesis of racemic guanacastepene A had been accomplished. However, in the absence of an authentic sample of the natural product, we sought to demonstrate with rigor that various epimers of guanacastepene A were indeed distinguishable by NMR spectroscopy. In this spirit, we prepared the remaining three diastereomers with respect to C5 and C13 (**1a**, **1b**, and **1c**).[22] Figure 1 reveals the diagnostic 1H NMR data of the various diastereomers. In short, the C13 protons in the epimeric 13-acetoxy compounds exhibit drastically different coupling constants. Interestingly, the epimeric C5 hydroxyls affect the peak shape of the aldehyde signal. This may reflect differing configurationally sensitive proclivities of the C5 hydroxyl groups for hydrogen bonding to the proximal formyl oxygen atom.

[Figure 1. Structures and NMR of four epimers]

δ = 9.91 (br s)

δ = 5.48 (d, J = 6.5 Hz)

15

5

AcO

O

OH

H''' 13

1

(*guanacastepene A*)

δ = 9.94 (s)

δ = 5.44 (d, J = 6.3 Hz)

15

5

AcO

O

OH

H''' 13

1a

natural guanacastepene A:
C13-H: δ = 5.48 (d, J = 7.0 ±0.5 Hz)
C15-H: δ = 9.91 (br s)

δ = 9.95 (br s)

δ = 5.44 (d, J = 12.0 Hz)

15

5

AcO

O

OH

H'' 13

1b

δ = 9.95 (s)

δ = 5.45 (d, J = 12.8 Hz)

15

5

AcO

O

OH

H'' 13

1c
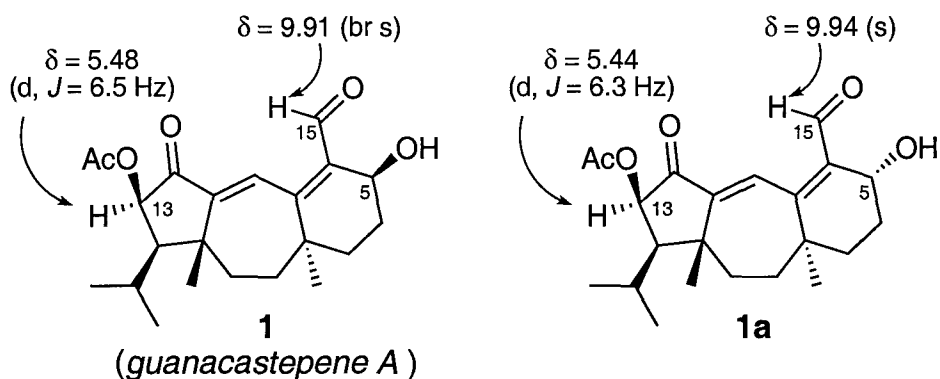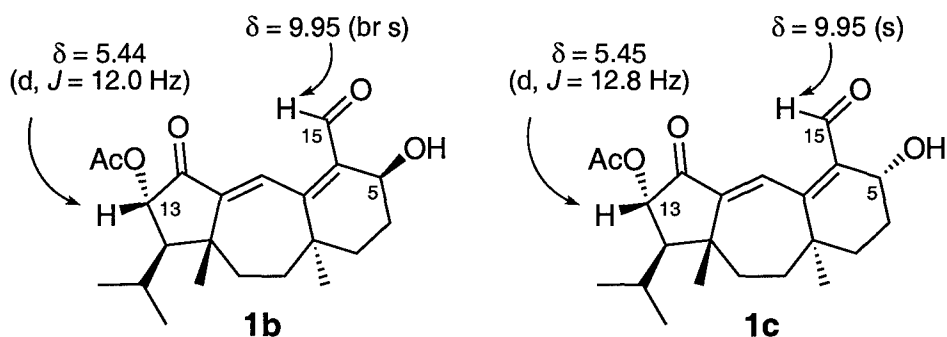
In the light of the observed β-face oxidation of ketone **7**, it was appropriate to revisit our original thinking about the steric biases of this system. Calculations using the MM2 force field[23] provided energy-minimized conformers of the silyl enol ether derived from **7** (**18**), one of which is shown in Figure 2. Based on these calculations, it appeared that the isopropyl group might not exert a dominant steric influence on facial selectivity. Furthermore, carbons 17 and 10 are approximately equidistant from the locus of the C13–C14 bond. By the same token, the modeling exercise did not reveal a

11

convincing steric bias to rationalize the highly selective
β-face epoxidation observed above.[24]

[Figure 2. energy minimized structures]
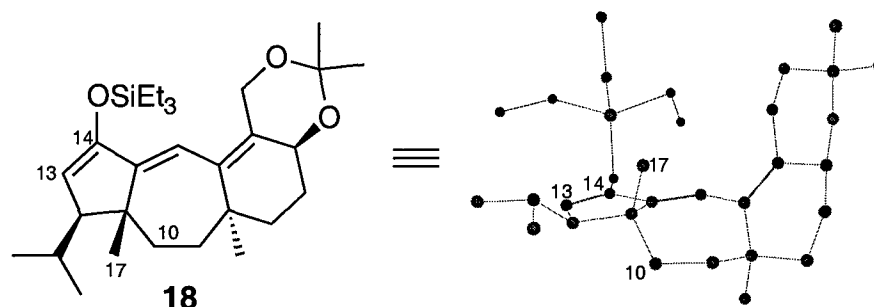
In summary, the total synthesis of the naturally
occurring guanacastepene A has been accomplished. Given the
absence of a comparison sample of the natural product, we
could be confident that this result had been achieved only
after the fashioning of the four-component library of
guanacastepene isomers wherein the stereogenic centers at
carbons 5 and 13 are independently permuted. These studies
should serve to underscore the subtlety of biases that
control stereochemical outcomes. Full details of the total
synthesis and a fuller airing of this fascinating
epoxidation tendency will be described shortly.

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[1] D. S. Tan, G. B. Dudley, S. J. Danishefsky, previous
communication in this issue.
[2] For the isolation and characterization of guanacastepene A
and related natural products, see: (a) Brady, S. F.; Singh,
M. P.; Janso, J. E.; Clardy, J. *J. Am. Chem. Soc.* **2000**,
122, 2116-2117; (b) Singh, M. P.; Janso, J. E.; Luckman, S.
W.; Brady, S. F.; Clardy, J.; Greenstein, M.; Maiese, W. M.
J. Antibiot. **2000**, *53*, 256-261; (c) Brady, S. F.; Bondi, S.
M.; Clardy, J. *J. Am. Chem. Soc.* **2001**, *123*, 9900-9901.

[3] For alternative approaches to the synthesis of guanacastepene A, see: (a) Snider, B. B.; Hawryluk, N. A. *Org. Lett.* **2001**, *3*, 569–572; (b) Snider, B. B.; Shi, B. *Tetrahedron Lett.* **2001**, *42*, 9123–9126; (c) Magnus, P; Waring, M. J.; Ollivier, C.; Lynch, V. *Tetrahedron Lett.* **2001**, *42*, 4947–4950; (d) Mehta, G.; Umarye, J. D. *Org. Lett. ASAP article* published on the web March 7, 2002.

[4] For earlier accounts of our studies, see: (a) Dudley, G. B.; Danishefsky, S. J. *Org. Lett.* **2001**, *3*, 2399–2402; (b) Dudley, G. B.; Tan, D. S.; Kim, G.; Tanski, J. M.; Danishefsky, S. J. *Tetrahedron Lett.* **2001**, *42*, 6789–6791.

[5] (a) For a discussion of the general preference for axial hydride delivery to cyclohexenones, with leading references, see Davis, A. P. In *Methods in Organic Chemistry (Houben-Weyl) Stereoselective Synthesis*, Workbench Edition E21; Helmchen, G., Hoffmann, R. W., Mulzer, J., Schaumann, E., Eds.; Georg Thieme Verlag: New York, 1996; Vol. 7, pp 4034–4037. (b) For a closely related example, see ref 3b.

[6] Based on extensive literature precedent (see ref. 5), this reduction was assumed to proceed as indicated in Scheme 2. Confirmation of this tentative assignment was obtained upon conversion to the natural product.

[7] For a review of reductions using lithium trialkoxyaluminum hydrides, see: Málek, J. *Org. React.* **1985**, *34*, 1–317.

[8] Krishnamurthy, S.; Brown, H. C. *J. Org. Chem.* **1977**, *42*, 1197–1201.

[9] Gemal, A. L.; Luche, J.-L. *J. Am. Chem. Soc.* **1981**, *103*, 5454–5459.

[10] The epimeric diols (**5** and **6**) are both advanced along stereoconvergent pathways to ketone **7**.

[11] For a review of the Mitsunobu reaction, see: Hughes, D. L.; *Org. React.* **1992**, *42*, 335–656.

[12] (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156; (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899.

[13] The yield for this oxidation step increases from 50% to 90% in the presence of pyridine (3 equiv relative to Dess-Martin).

[14] (a) Zhu, Y.; Shu, L.; Tu, Y.; Shi, Y. *J. Org. Chem.* **2001**, *66*, 1818–1826; (b) Heathcock, C.; Smith S. C. *J. Org. Chem.* **1994**, *59*, 6828–6839.

[15] Brevet, J.-L.; Fournet, G.; Gore, J. *Synth. Commun.* **1996**, *26*, 4185–4194.

[16] The product mixture comprised acetoxy- and hydroxy-ketones. This crude material was immediately re-acetylated prior to characterization.

[17] (a) Rubottom, G. M.; Vazquez, M. A.; Pelegrina, D. R. *Tetrahedron Lett.* **1974**, 4319–4322; (b) Danishefsky, S. J.; Masters, J. J.; Young, W. B.; Link, J. T.; Snyder, L. B.; Magee, T. V.; Jung, D. K.; Isaacs, R. C. A.; Bornmann, W. G.; Alaimo, C. A.; Coburn, C. A.; DiGrandi, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2843–2859.

[18] Rubottom, G. M.; Gruber, J. M.; Boeckman, R. K., Jr.; Ramaiah, M.; Medwid, J. B. *Tetrahedron Lett.* **1978**, 4603–4606.

[19] De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. *J. Org. Chem.* **1997**, *62*, 6974–6977.

[20] An authentic sample is no longer available for comparison (J. Clardy, personal communication). In fact, at the present time chemical synthesis is the only source of this compound.

[21] ^{1}H NMR for synthetic guanacastepene at 25 °C (acetone-d_6, 400 MHz): δ 9.91 (br s, 1 H), 7.45 (d, J = 1.1 Hz, 1 H), 5.48 (d, J = 6.5 Hz, 1 H), 4.62 (m, 1 H), 3.97 (br s, 1 H) (OH), 2.08 (s, 3H), 1.99 (m), 1.90 (m), 1.79 (m), 1.63 (m), 1.40 (m), 1.27 (s, 3 H), 1.12 (d, J = 6.6 Hz, 3 H), 1.09 (s, 3 H), 0.93 (d, J = 6.4 Hz, 3 H). ^{1}H NMR for synthetic guanacastepene at –50 °C (acetone-d_6, 500 MHz, key signals): *Major* δ 9.96 (s), 7.42 (s), 5.45 (d, J = 5.6 Hz, 1 H), 4.64 (m, 1 H), 4.59 (d, J = 5.4 Hz) (OH), 2.10 (s, 3H); *Minor* δ 9.72 (s), 7.49 (s), 5.53 (d, J = 7.1 Hz, 1 H), 4.52 (m, 1 H), 4.48 (d, J = 4.1 Hz) (OH), 2.11 (s, 3H). These data match the data obtained directly from the ^{1}H NMR spectra of the natural product. We are grateful to Prof. Jon Clardy and Sean Brady for providing detailed NMR spectra of natural guanacastepene A.

[22] **1a** and **1c** were prepared from **14** and **15**, respectively, by analogy to the conversion of **17** → **1** (Scheme 6). **1b** was similarly derived from the minor diastereomer obtained in the Rubottom-type oxidation of **7** (Scheme 5).

[23] MacroModel version 5.5, Department of Chemistry, Columbia University.

[24] The possibility that the silyl group orients itself preferentially on the α-face, blocking the epoxidation from below, was also considered. However, the MM2 calculations suggest little energy difference with respect to various silyl group orientations, disfavoring this hypothesis.

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PROVISIONAL APPLICATION TRANSMITTAL
(REQUEST FOR FILING A PROVISIONAL APPLICATION FOR PATENT UNDER 37 CFR § 1.53(C))

Dear Sir:

Please find enclosed a patent application and papers as follows for:

Inventor(s):

Given Name (first and middle)	Family Name or Surname	Residence (City and State or Foreign Country)
Samuel J.	Danishefsky	Englewood, NJ
Songnian	Lin	New York, NY

Title of the Invention: *Cyclic Peptides, Analogues and Uses Thereof*

A) ENCLOSED APPLICATION PARTS:

1) _X_ Specification   PAGES: <u>48</u>

2) _X_ Claims   PAGES: <u>5</u>

3) _X_ Abstract   PAGES: <u>1</u>

4) ___ Drawing(s)   SHEETS: _____

               TOTAL: <u>54</u>

B) OTHER ACCOMPANYING APPLICATION PARTS:

    3) _X_ Return Receipt Postcard (MPEP § 503) (specifically itemized)

    4) ___ Application Data Sheet. See 37 CFR § 1.76

    5) ___ **OTHER:** (if applicable, specified below)

D) CORRESPONDENCE ADDRESS:

    _X_ Customer Bar Code Label: _____24280_____

                               PATENT TRADEMARK OFFICE

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E) METHOD OF PAYMENT OF FILING FEES:

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    _X_ The Commissioner is hereby authorized to charge filing fees or credit any
        overpayment to Deposit Account Number: 03-1721.

    FILING FEE AMOUNT ($): 80.00

The present invention was made with support from the National Cancer Institute Core Grant No. 08748 (CA 28824-23) and grant from the U.S. Army to Dr. Songnian Lin (Grant No.: F32 USA-DAMD-17-99-9373). Therefore, the government has certain rights in this invention.

Respectfully Submitted,

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CYCLIC PEPTIDES, ANALOGUES AND USES THEREOF

GOVERNMENT SUPPORT

10   ## BACKGROUND OF THE INVENTION

TMC-95A (**1**) and its diastereomers, TMC-95B-D (**2-4**, Figure 1), are cyclic peptides recently isolated as soil sample derived fermentation products of *Apiospora montagnei* Sacc. TC1093 (*See* Y. Koguchi, J. Kohno, M. Nishio, K. Takahashi, T. Okuda, T. Ohnuki, S.

15   Komatsubara, *J. Antibiot.* **2000**, *53*, 105-109; J. Kohno, Y. Koguchi, M. Nishio, K. Nakao, M. Kuroda, R. Shimizu, T. Ohnuki, S. Komatsubara, *J. Org. Chem.* **2000**, *65*, 990-995).

TMC-95	R^1	R^2	R^3	R^4
A (1)	H	OH	CH_3	H
B (2)	H	OH	H	CH_3
C (3)	OH	H	CH_3	H
D (4)	OH	H	H	CH_3
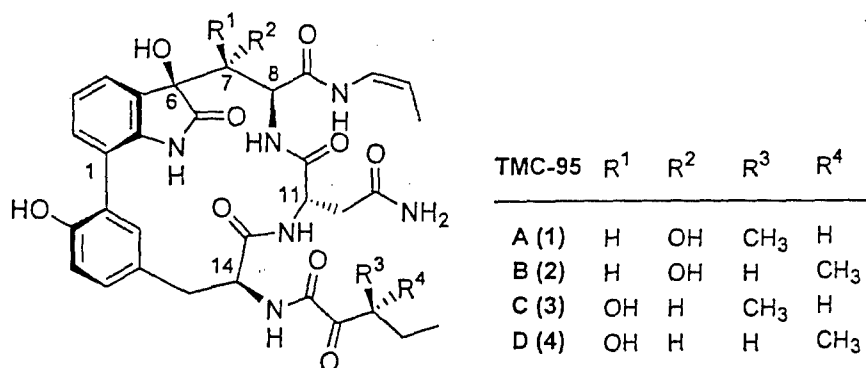
20   **Figure 1**: Structures of TMC-95A, B, C, and D (**1-4**).

Biological studies (*See* Y. Koguchi, J. Kohno, M. Nishio, K. Takahashi, T. Okuda, T. Ohnuki, S. Komatsubara, *J. Antibiot.* **2000**, *53*, 105-109) have shown that TMC-95A inhibited the chymotrypsin like (ChT-L), trypsin like (T-L) and peptidoglutamyl hydrolyzing (PGPH)

25   activities of the 20S proteasome (*See* A. Ciechanover, A. L. Schwartz, *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2727-2730; M. Orlowski, C. Michaud, *Biochemistry* **1989**, *28*, 9270-9278) with IC_{50} values of 5.4 nM, 200 nM and 60 nM respectively. TMC-95B inhibited these

activities to the same extent as TMC-95A, while TMC-95C and D were 20 to 150 times weaker. TMC-95A has also shown cytotoxic activities against HCT-116 (human colon carcinoma cells) and HL-60 (human promyelocytic leukemia cells) with IC_{50} values of 4.4 μM and 9.8 μM, respectively. These four diastereomers are structurally characterized as novel cyclic peptides containing L-tyrosine, L-asparagine, highly oxidized L-tryptophan, (Z)-1-propenylamine, and 3-methyl-2-oxopentanoic acid moieties. Although the phenyl-indole ring attachment is found in a few natural products such as chloropeptin (K. Matsuzaki; H. Ikeda; T. Ogino; A. Matsumoto; H.B. Woodruf; H. Tanaka; S. Omura *J. Antibiot.* **1994**, *47*, 1173-1174), complestatin (I. Kaneko, K. Kamoshida, S. Takahashi, *J. Antibiot.* **1989**, *42*, 236-241), diazonamide (N. Lindquist, W. Fenical, G. D. Van Duyne, J. Clardy, *J. Am. Chem. Soc.* **1991**, *113*, 2303-4, and the kistamicins ((a) N. Naruse, O. Tenmyo, S. Kobaru, M. Hatori, K. Tomita, Y. Hamagishi, T. Oki, J. Careofokumura, *J. Antibiot.* **1993**, *46*, 1804-1811; (b). N. Naruse, M. Oka, M. Konishi, T. Oki. *J. Antibiot.* **1993**, *46*,1812-1818), the presence of an oxindole ring in such macrocyclic context is apparently rare. The structural novelty of the TMC compounds and the biological issues they raise prompted our laboratory to embark on a program directed to their total synthesis.

The ability of these natural products and other compounds (e.g., boronic acids and esters, peptide aldehydes, α',β'-epoxyketones and vinyl sulfones to name a few, *see*, for example, Adams *et al. Cancer Res.* **1999**, *59*, 2615-2622; Rock *et al. Cell* **1994**, *78*, 761-771; Lynas *et al. Bioorg. Med. Chem. Lett.* **1998**, *8*, 373-378; Spaltenstein *et al. Tet. Lett.* **1996**, *37*, 1343-1346; Bogyo *et al. Chem. Biol.* **1998**, *5*, 307-320; and generally Myung *et al. Medicinal Research Reviews* **2001**, *21*, 245-273) to act as proteasome inhibitors has attracted significant interest because of the wide range of cellular substrates and processes controlled by the ubiquitin-proteasome pathway. For example, the oscillation of cyclins (cell cycle proteins required for the orderly progression through the cell cycle) has been found to be due to the regulated degradation mediated by the ubiquitin-proteasome pathway, and inhibition of this pathway is believed to result in the blockage of cell cycle progression. Additionally, the transcriptional factor NF-κB is another regulatory protein involved in a variety of cellular processes including immune and inflammatory responses, apoptosis, and cellular proliferation, whose mode of action is controlled by the ubiquitin-proteasome pathway. Furthermore, it has also been shown that the ubiquitin-proteasome pathway is involved in retrovirus assembly and

thus may be useful for the development of anti-HIV drugs. For a general discussion of the ubiquitin-proteasome pathway and proteasome inhibitors <u>see</u>, Myung *et al.* "The Ubiquitin-Proteasome Pathway and Proteasome Inhibitors" *Medicinal Research Reviews* **2001**, *21*, 245-273.

5   Clearly, it would be desirable to investigate additional classes of compounds for use as proteasome inhibitors and more generally as therapeutic agents. In particular, it would be desirable to investigate those compounds having novel core structures such as TMC-95A and its diastereomers.

10   *DESCRIPTION OF THE INVENTION*

As discussed above, the demonstrated antitumor activity of the natural products TMC-95A (1) and its diastereomers, as well as their ability to inhibit activities of the 20S proteasome, has led to increased interest in the synthesis and biological investigation of these compounds. In recognition of the need to further develop the therapeutic potential of this class

15   of compounds, the present invention provides novel cyclic peptides. In certain embodiments, the compounds of the present invention can be used for the treatment of cancer and inflammatory disorders. More generally, in certain other embodiments, the compounds of the invention act as proteasome inhibitors.

20   *1) General Description of Compounds of the Invention*

The compounds of the invention include compounds of the general formula (I) as further defined below:
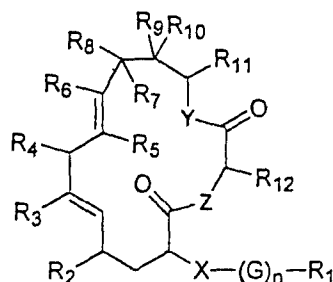
25   (I)

and pharmaceutically acceptable derivatives thereof;

wherein R_1 is hydrogen, $-CH_2Cl$, $-CH_2Ac$, $-C=C-S(O)_2-CH_3$, $B(OR_{1a})_2$, $-(C=O)R_{1a}$, or

an aliphatic, heteroaliphatic, aryl or heteroaryl moiety, or [epoxide structure with O] or [aziridine structure with $N-R_{1a}$] ,wherein each occurrence of R_{1a} is hydrogen, an oxygen protecting group, or an aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;
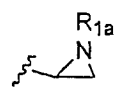
5   each occurrence of G is independently $(C=O)$ or $-CH_2-$;

n is 0, 1, 2, or 3;

X is NH, O, CH_2, or is absent;

Y and Z are each independently NH, N(alkyl), O, S, CH_2, CH(alkyl), or C(alkyl)(alkyl);

R_2 and R_3 are each hydrogen, or an aliphatic, heteroaliphatic, aryl, or heteroaryl moiety,

10   or wherein R_2 and R_3 taken together are an aryl, heteroaryl, cycloaliphatic, or heterocycloaliphatic moiety;

R_4, R_5, R_6 and R_7 are each hydrogen, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety, or wherein R_4 and R_6 taken together are an aryl, heteroaryl, cycloaliphatic, or heterocycloaliphatic moiety, and wherein R_5 and R_7, taken together, are an aryl, heteroaryl,

15   cycloaliphatic or heterocycloaliphatic moiety;

R_8, R_9 and R_{10} are each independently hydrogen, protected or unprotected hydroxyl, protected or unprotected thio, protected or unprotected amino, acyl, acyloxy, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety;

R_{11} is hydrogen, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkoxycarbonyl,

20   alkylaminocarbonyl, or alkenylaminocarbonyl moiety or is (alipahtic)OR_{11a}, or (heteroaliphatic)OR_{11a}, wherein R_{11a} is hydrogen, a protecting group, acyl, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety;

R_{12} is hydrogen, an aliphatic, heteroaliphatic, aryl, or heteroaryl moiety, alkoxycarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, or $-(alkyl)(C=O)NH_2$,

25   whereby each of the foregoing aliphatic and heteroaliphatic moieties is independently substituted or unsubstituted, cyclic or acyclic, linear or branched, and each of the foregoing aryl and heteroaryl moieties is independently substituted or unsubstituted.

It will be appreciated that for compounds as generally described above, certain classes of compounds are of special interest. For example, one class of compounds of special interest includes those compounds having the structure:
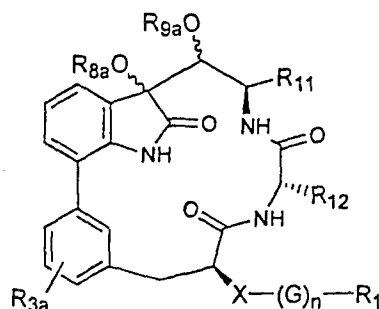
wherein R_{3a} is hydrogen, OR_{3b}, $-N(R_{3b})_2$, SR_{3b}, halogen, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety, wherein each occurrence of R_{3b} is independently hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_1, R_{11}, R_{12}, X, G and n are each defined as above and in classes and subclasses herein.

Another class of compounds of special interest consists of compounds having the structure:
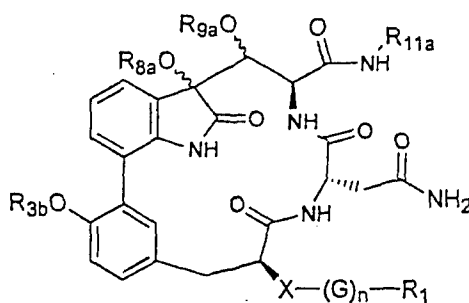
wherein R_{3b} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{11a} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_1, R_{12}, X, G and n are each defined as above and in classes and subclasses herein.

Another class of compounds of special interest consists of compounds having the structure:

5

wherein R_{3b} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{11a} is hydrogen, a protecting group, or an

10 aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_1, is defined as above and in classes and subclasses herein.

Another class of compounds of special interest consists of compounds having the structure:

15

wherein R_{3b} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; R_{11a} is hydrogen, a protecting group, or an

20 aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_1, is defined as above and in classes and subclasses herein.

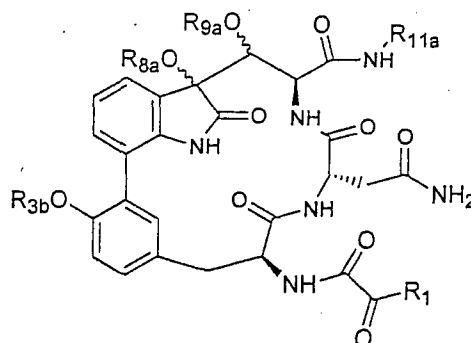A number of important subclasses of each of the foregoing classes deserve separate mention; these subclasses include subclasses of the foregoing classes in which:

5   i)   compounds, pharmaceutical compositions and methods of treating disorders as described above and herein include compounds as described generally herein, but in certain embodiments exclude TMC-95A, B, C and D;

ii)   compounds, pharmaceutical compositions and methods of treating disorders as described herein include compounds as described generally herein, but in certain embodiments

10   the compounds are purified compounds;

iii)   Y and Z are each NH;

iv)   X is NH, n is 2, each occurrence of G is (C=O), and R_1 is lower alkyl or lower alkenyl;

v)   X is NH, n is 1, G is (C=O) and R_1 is lower alkyl or lower alkoxy;

vi) R_{11} is (C=O)NHR_{11a} where R_{11a} is lower alkyl or lower alkenyl; and

15   vii) R_{12} is -CH_2(C=O)NH_2;

viii) R_{11} is CH_2OR_{11a}, wherein R_{11a} is hydrogen, a protecting group, acyl, or lower alkyl.

Some of the foregoing compounds can exist in various isomeric forms, e.g., stereoisomers and/or diastereomers. Furthermore, certain compounds, as described herein may

20   have one or more double bonds that can exist as either the Z or E isomer, unless otherwise indicated. The invention additionally encompasses the compounds as individual isomers substantially free of other isomers and alternatively, as mixtures of various isomers, e.g., racemic mixtures of stereoisomers. In addition to the above-mentioned compounds per se, this invention also encompasses pharmaceutically acceptable derivatives of these compounds and

25   compositions comprising one or more compounds of the invention and one or more pharmaceutically acceptable excipients or additives.

Compounds of this invention which are of particular interest include those which:

30   • exhibit activity generally as inhibitors of proteasome activity;

- exhibit an antiproliferative and/or anticancer effect on suitable cell lines maintained in vitro, or in animal studies using a scientifically acceptable model;

- exhibit an anti-inflammatory effect on suitable cell lines maintained in vitro, or in animal studies using a scientifically acceptable model;

5
- exhibit in vivo efficacy vs. human cancer xenografts;

- exhibit a favorable therapeutic profile (e.g., safety, efficacy, and stability);

- exhibit inhibition of cyclin degradation;

- exhibit inhibition of HIV replication; and

- exhibit inhibition of cytolytic immune responses.

10

As discussed above, certain of the compounds as described herein exhibit activity generally as proteasome inhibitors. More specifically, compounds of the invention demonstrate anti-cancer activity and thus the invention further provides a method for treating cancer. The method involves the administration of a therapeutically effective amount of the

15   compound or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited to a human or animal) in need of it. In certain embodiments, the inventive compounds are also useful for the treatment of inflammatory disorders, HIV, or for the treatment of other proliferative disorders. These disorders include, but are not limited to cancer, psoriasis, arthritis, sepsis, AIDS, and organ graft rejection to name a few.

20

3) Compounds and Definitions

As discussed above, this invention provides novel compounds with a range of biological properties. Compounds of this invention have biological activities relevant for the treatment of cancer and/or inflammatory disorders, and/or disorders caused by activation of

25   certain regulatory subunits of the proteasome.

Compounds of this invention include those specifically set forth above and described herein, and are illustrated in part by the various classes, subgenera and species disclosed elsewhere herein.

It will be appreciated by one of ordinary skill in the art that asymmetric centers may

30   exist in the compounds of the present invention. Thus, inventive compounds and pharmaceutical compositions thereof may be in the form of an individual enantiomer,

diastereomer or geometric isomer, or may be in the form of a mixture of stereoisomers. Furthermore, it will be appreciated that certain of the compounds disclosed herein contain one or more double bonds and these double bonds can be either Z or E, unless otherwise indicated. In certain embodiments, the compounds of the invention are enantiopure compounds. In certain other embodiments, a mixture of stereoisomers or diastereomers are provided.

Additionally, the present invention provides pharmaceutically acceptable derivatives of the inventive compounds, and methods of treating a subject using these compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents. The phrase, "pharmaceutically acceptable derivative", as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety that is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester which is cleaved *in vivo* to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.

Certain compounds of the present invention, and definitions of specific functional groups are also described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference. Furthermore, it will be appreciated by one of ordinary skill in the art that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group", has used herein, it is meant that a particular functional moiety,

e.g., O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen and carbon protecting groups may be utilized. For example, in certain embodiments, as detailed herein, certain exemplary oxygen protecting groups are utilized. These oxygen protecting groups include, but are not limited to methyl ethers, substituted methyl ethers (e.g., MOM (methoxymethyl ether), MTM (methylthiomethyl ether), BOM (benzyloxymethyl ether), PMBM (p-methoxybenzyloxymethyl ether), to name a few), substituted ethyl ethers, substituted benzyl ethers, silyl ethers (e.g., TMS (trimethylsilyl ether), TES (triethylsilylether), TIPS (triisopropylsilyl ether), TBDMS (t-butyldimethylsilyl ether), tribenzyl silyl ether, TBDPS (t-butyldiphenyl silyl ether), to name a few), esters (e.g., formate, acetate, benzoate (Bz), trifluoroacetate, dichloroacetate, to name a few), carbonates, cyclic acetals and ketals. In certain other exemplary embodiments, nitrogen protecting groups are utilized. These nitrogen protecting groups include, but are not limited to, carbamates (including methyl, ethyl and substituted ethyl carbamates (e.g., Troc), to name a few) amides, cyclic imide derivatives, N-Alkyl and N-Aryl amines, imine derivatives, and enamine derivatives, to name a few. Certain other exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the present invention. Additionally, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this

invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated

5   to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of

10   the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment, for example of inflammatory disorders, cancer, and other disorders, as described generally above. The term "stable", as used herein, preferably refers to

15   compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (i.e., unbranched), branched, cyclic, or polycyclic aliphatic hydrocarbons, which are

20   optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. Thus, as used herein, the term "alkyl" includes straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used

25   herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the

30   invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In

yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4

5   carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, -CH$_2$-cyclopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, cyclobutyl, -CH$_2$-cyclobutyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, cyclopentyl, -CH$_2$-cyclopentyl-n, hexyl, sec-hexyl, cyclohexyl, -CH$_2$-cyclohexyl moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not

10   limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

The term "alkoxy" (or "alkyloxy"), or "thioalkyl" as used herein refers to an alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom

15   or through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4

20   aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

The term "alkylamino" refers to a group having the structure -NHR'wherein R' is alkyl,

25   as defined herein. The term "dialkylamino" refers to a group having the structure –N(R')$_2$, wherein R' is alkyl, as defined herein. The term "aminoalkyl" refers to a group having the structure NH$_2$R'-, wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and

30   alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments,

the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

Some examples of sutstituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO$_2$; -CN; -CF$_3$; -CH$_2$CF$_3$; -CHCl$_2$; -CH$_2$OH; -CH$_2$CH$_2$OH; -CH$_2$NH$_2$; -CH$_2$SO$_2$CH$_3$; -C(O)R$_x$; -CO$_2$(R$_x$); -CON(R$_x$)$_2$; -OC(O)R$_x$; -OCO$_2$R$_x$; -OCON(R$_x$)$_2$; -N(R$_x$)$_2$; -S(O)$_2$R$_x$; -NR$_x$(CO)R$_x$ wherein each occurrence of R$_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. Substituents include, but are not limited to, any of the previously mentioned substitutents, i.e., the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. In certain embodiements of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one or more of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl;

5   alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO$_2$; -CN; -CF$_3$; -CH$_2$CF$_3$; -CHCl$_2$; - CH$_2$OH; -CH$_2$CH$_2$OH; -CH$_2$NH$_2$; -CH$_2$SO$_2$CH$_3$; -C(O)R$_x$; -CO$_2$(R$_x$); -CON(R$_x$)$_2$; -OC(O)R$_x$; - OCO$_2$R$_x$; -OCON(R$_x$)$_2$; -N(R$_x$)$_2$; -S(O)$_2$R$_x$; -NR$_x$(CO)R$_x$ wherein each occurrence of R$_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl,

10   alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments

15   shown in the Examples that are described herein.

The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic or hetercyclic moieties, may optionally be substituted

20   with substituents including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO$_2$; -CN; -CF$_3$; -CH$_2$CF$_3$; -CHCl$_2$; - CH$_2$OH; -CH$_2$CH$_2$OH; -CH$_2$NH$_2$; -CH$_2$SO$_2$CH$_3$; -C(O)R$_x$; -CO$_2$(R$_x$); -CON(R$_x$)$_2$; -OC(O)R$_x$; - OCO$_2$R$_x$; -OCON(R$_x$)$_2$; -N(R$_x$)$_2$; -S(O)$_2$R$_x$; -NR$_x$(CO)R$_x$ wherein each occurrence of R$_x$

25   independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional

30   examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

The term "heteroaliphatic", as used herein, refers to aliphatic moieties which contain one or more oxygen sulfur, nitrogen, phosphorus or silicon atoms, e.g., in place of carbon atoms. Heteroaliphatic moieties may be branched, unbranched, cyclic or acyclic and include saturated and unsaturated heterocycles such as morpholino, pyrrolidinyl, etc. In certain

5   embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO$_2$; -CN; -CF$_3$; -CH$_2$CF$_3$; -CHCl$_2$; -CH$_2$OH; -CH$_2$CH$_2$OH; -CH$_2$NH$_2$; -CH$_2$SO$_2$CH$_3$; -

10   C(O)R$_x$; -CO$_2$(R$_x$); -CON(R$_x$)$_2$; -OC(O)R$_x$; -OCO$_2$R$_x$; -OCON(R$_x$)$_2$; -N(R$_x$)$_2$; -S(O)$_2$R$_x$; -NR$_x$(CO)R$_x$ wherein each occurrence of R$_x$ independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein

15   any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

20   The term "haloalkyl" denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

The term "heterocycloalkyl" or "heterocycle", as used herein, refers to a non-aromatic 5-, 6- or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic

25   group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to a

30   benzene ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl,

oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl. In certain embodiments, a "substituted heterocycloalkyl or heterocycle" group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one or more of the hydrogen atoms thereon with but are not

5 limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; - OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; - $CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; - $S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited

10 to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substitutents described above and herein may be substituted or unsubstituted. Additional examples or generally applicable substituents are illustrated by the

15 specific embodiments shown in the Examples which are described herein.

4) Uses, Formulation and Administration

Pharmaceutical Compositions

20 As discussed above this invention provides novel compounds that have biological properties useful for the treatment of cancer, inflammatory disorders and other disorders caused by regulatory units of the proteasome. In certain embodiments of special interest, the inventive compounds as useful for the treatment of cancer.

Accordingly, in another aspect of the present invention, pharmaceutical compositions

25 are provided, which comprise any one of the compounds described herein (or a prodrug, pharmaceutically acceptable salt or other pharmaceutically acceptable derivative thereof), and optionally comprise a pharmaceutically acceptable carrier. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents. Alternatively, a compound of this invention may be administered to a patient in need thereof in

30 combination with the administration of one or more other therapeutic agents. For example, additional therapeutic agents for conjoint administration or inclusion in a pharmaceutical

composition with a compound of this invention may be an anti-cancer agent, anti-inflammatory, or anti-HIV agent approved for the treatment of cancer, inflammatory disorders or HIV to name a few, as discussed in more detail herein, or it may be any one of a number of agents undergoing approval in the Food and Drug Administration that ultimately obtain

5   approval for the treatment of any of these disorders or other disorders caused by certain regulatory subunits of the proteasome. It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present invention, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically

10   acceptable salts, esters, salts of such esters, or a prodrug or other adduct or derivative of a compound of this invention which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which

15   are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts of amines, carboxylic acids, and other types of compounds, are well known in the art. For example, S.M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in *J.*

20   *Pharmaceutical Sciences, 66*: 1-19 (1977), incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting a free base or free acid function with a suitable reagent, as described generally below. For example, a free base function can be reacted with a suitable acid. Furthermore, where the compounds of the invention carry an acidic moiety, suitable

25   pharmaceutically acceptable salts thereof may, include metal salts such as alkali metal salts, e.g. sodium or potassium salts; and alkaline earth metal salts, e.g. calcium or magnesium salts. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid,

30   maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate,

alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate,

5   lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the

10   like.   Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Additionally, as used herein, the term "pharmaceutically acceptable ester" refers to

15   esters that hydrolyze in vivo and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include

20   formates, acetates, propionates, butyrates, acrylates and ethylsuccinates.

Furthermore, the term "pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the issues of humans and lower animals with undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable

25   benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formula, for example by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche,

30   ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference.

As described above, the pharmaceutical compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders,

5   lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable

10   biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium

15   carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatine; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil, sesame oil; olive oil; corn oil and soybean oil; glycols; such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogenfree water; isotonic

20   saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

25

Uses of Compounds of the Invention

As described in more detail herein, in general, the present invention provides compounds useful for the treatment of cancer and inflammatory disorders. Without wishing to be bound by any particular theory, more generally, the compounds of the invention have also

30   been shown to act as inhibitors of the proteasome function and thus also are useful generally for the treatment of disorders caused by certain regulatory subunits of the proteasome.

For example, the compounds described above and in classes and subclasses herein can be used to treat chronic or acute inflammation that is the result of transplantation rejection, arthritis, rheumatoid arthritis, infection, dermatosis, inflammatory bowel disease, asthma, osteoporosis, osteoarthritis and autoimmune disease. Additionally, inflammation associated

5   with psoriasis and restenosis can also be treated.

The term "treatment of inflammation" or "treating inflammation" is intended to include the administration of compounds of the present invention to a subject for purposes which can include prophylaxis, amelioration, prevention or cure of an inflammatory response. Such treatment need not necessarily completely ameliorate the inflammatory response. Further, such

10   treatment can be used in conjunction with other traditional treatments for reducing the inflammatory condition known to those of skill in the art.

The proteasome inhibitors of the invention can be provided as a "preventive" treatment before detection of an inflammatory state, so as to prevent the same from developing in patients at high risk for the same, such as, for example, transplant patients.

15   In another embodiment, efficacious levels of the proteasome inhibitors of the invention are administered so as to provide therapeutic benefits against the secondary harmful inflammatory effects of inflammation. By an "efficacious level" of a composition of the invention is meant a level at which some relief is afforded to the patient who is the recipient of the treatment. By an "abnormal" host inflammatory condition is meant an level of

20   inflammation in the subject at a site which exceeds the norm for the healthy medical state of the subject, or exceeds a desired level. By "secondary" tissue damage or toxic effects is meant the tissue damage or toxic effects which occur to otherwise healthy tissues, organs, and the cells therein, due to the presence of an inflammatory response, including as a result of a "primary" inflammatory response elsewhere in the body.

25   Amounts and regimens for the administration of proteasome inhibitors and compositions of the invention can be determined readily by those with ordinary skill in the clinical art of treating inflammation-related disorders such as arthritis, tissue injury and tissue rejection. Generally, the dosage of the composition of the invention will vary depending upon considerations such as: type of pharmaceutical composition employed; age; health; medical

30   conditions being treated; kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired; extent of tissue damage; gender; duration of the symptoms; and,

counter indications, if any, and other variables to be adjusted by the individual physician. A desired dosage can be administered in one or more applications to obtain the desired results. Pharmaceutical compositions containing the proteasome inhibitors of the invention can be provided in unit dosage forms.

5   Thus, the proteasome inhibitors are useful for treating such conditions as tissue rejection, arthritis, local infections, dermatoses, inflammatory bowel diseases, autoimmune diseases, etc. The proteasome inhibitors of the present invention can be employed to prevent the rejection or inflammation of transplanted tissue or organs of any type, for example, heart, lung, kidney, liver, skin grafts, and tissue grafts.

10   Compounds of the present invention inhibit the growth of cancer cells. Thus, the compounds can be employed to treat cancer, psoriasis, restenosis or other cell proliferative diseases in a patient in need thereof.

By the term "treatment of cancer" or "treating cancer" is intended description of an activity of compounds of the present invention wherein said activity prevents or alleviates or

15   ameliorates any of the specific phenomena known in the art to be associated with the pathology commonly known as "cancer." The term "cancer" refers to the spectrum of pathological symptoms associated with the initiation or progression, as well as metastasis, of malignant tumors. By the term "tumor" is intended, for the purpose of the present invention, a new growth of tissue in which the multiplication of cells is uncontrolled and progressive. The tumor

20   that is particularly relevant to the invention is the malignant tumor, one in which the primary tumor has the properties of invasion or metastasis or which shows a greater degree of anaplasia than do benign tumors.

Thus,"treatment of cancer" or "treating cancer" refers to an activity that prevents, alleviates or ameliorates any of the primary phenomena (initiation, progression, metastasis) or

25   secondary symptoms associated with the disease. Cancers that are treatable are broadly divided into the categories of carcinoma, lymphoma and sarcoma. Examples of carcinomas that can be treated by the composition of the present invention include, but are not limited to: adenocarcinoma, acinic cell adenocarcinoma, adrenal cortical carcinomas, alveoli cell carcinoma, anaplastic carcinoma, basaloid carcinoma, basal cell carcinoma, bronchiolar

30   carcinoma, bronchogenic carcinoma, renaladinol carcinoma, embryonal carcinoma, anometroid carcinoma, fibrolamolar liver cell carcinoma, follicular carcinomas, giant cell carcinomas,

hepatocellular carcinoma, intraepidermal carcinoma, intraepithelial carcinoma, leptomanigio carcinoma, medullary carcinoma, melanotic carcinoma, menigual carcinoma, mesometonephric carcinoma, oat cell carcinoma, squamal cell carcinoma, sweat gland carcinoma, transitional cell carcinoma, and tubular cell carcinoma. Sarcomas that can be treated by the composition of

5   the present invention include, but are not limited to: amelioblastic sarcoma, angiolithic sarcoma, botryoid sarcoma, endometrial stroma sarcoma, ewing sarcoma, fascicular sarcoma, giant cell sarcoma, granulositic sarcoma. immunoblastic sarcoma, juxaccordial osteogenic sarcoma, coppices sarcoma, leukocytic sarcoma (leukemia), lymphatic sarcoma (lympho sarcoma), medullary sarcoma, myeloid sarcoma (granulocitic sarcoma), austiogenci sarcoma,

10   periosteal sarcoma, reticulum cell sarcoma (histiocytic lymphoma), round cell sarcoma, spindle cell sarcoma, synovial sarcoma, and telangiectatic audiogenic sarcoma. Lymphomas that can be treated by the composition of the present invention include, but are not limited to: Hodgkin's disease and lymphocytic lymphomas, such as Burkitt's lymphoma, NPDL, NML, NH and diffuse lymphomas.

15   Thus, as described above, in another aspect of the invention, a method for the treatment of cancer, inflammatory disorders, and other disorders caused by certain regulatory subunits of the proteasome, is provided comprising administering a therapeutically effective amount of a compound of formula (I), and classes and subclasses thereof, as described herein, to a subject in need thereof. It will be appreciated that the compounds and compositions, according to the

20   method of the present invention, may be administered using any amount and any route of administration effective for the treatment of the disorders described generally above. It will be appreciated that the exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular therapeutic agent, its mode of administration, and the like. The compounds of the invention

25   are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of therapeutic agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific

30   therapeutically effective dose level for any particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the

activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

Furthermore, after formulation with an appropriate pharmaceutically acceptable carrier in a desired dosage, the pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered at dosage levels of about 0.001 mg/kg to about 50 mg/kg, from about 0.01 mg/kg to about 25 mg/kg, or from about 0.1 mg/kg to about 10 mg/kg of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect. It will also be appreciated that dosages smaller than 0.001 mg/kg or greater than 50 mg/kg (for example 50-100 mg/kg) can be administered to a subject. In certain embodiments, compounds are administered orally or parenterally.

Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or

solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic

5   mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable

10   medium prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension or crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon

15   crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be

20   controlled. Examples of other biodegradable polymers include (poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating

25   excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert,

30   pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic

acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar--agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption

5   accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

10   Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain

15   opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular

20   weight polethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid

25   dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose and starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain

30   opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner.

Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms are made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

It will also be appreciated that the compounds and pharmaceutical compositions of the present invention can be formulated and employed in combination therapies, that is, the compounds and pharmaceutical compositions can be formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another anti-inflammatory agent or anticancer agent), or they may achieve different effects (e.g., control of any adverse effects).

TREATMENT KITS

In other embodiments, the present invention relates to a kit for conveniently and effectively carrying out the methods in accordance with the present invention. In general, the pharmaceutical pack or kit comprises one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Such kits are especially suited for the delivery of solid oral forms such as tablets or capsules. Such a kit preferably includes a number of unit dosages, and may also include a card having the dosages oriented in

the order of their intended use. If desired, a memory aid can be provided, for example in the form of numbers, letters, or other markings or with a calendar insert, designating the days in the treatment schedule in which the dosages can be administered. Alternatively, placebo dosages, or calcium dietary supplements, either in a form similar to or distinct from the

5   dosages of the pharmaceutical compositions, can be included to provide a kit in which a dosage is taken every day. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

10

EQUIVALENTS

The representative examples that follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in

15   addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art.

20   The following examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

EXEMPLIFICATION

25   The compounds of this invention and their preparation can be understood further by the examples that illustrate some of the processes by which these compounds are prepared or used. It will be appreciated, however, that these examples do not limit the invention. Variations of the invention, now known or further developed, are considered to fall within the scope of the present invention as described herein and as hereinafter claimed.

30

1) Synthesis of Exemplary Compounds:

As described above, the present invention provides novel cyclic peptides, certain of which are based on the proteasome inhibitors TMC-95A and its diastereomers TMC-95B, C and D.

Example 1: Synthesis of Proteasome Inhibitors TMC-95A and B

In one embodiment of interest, compounds having the unique cyclic core of TMC-95A are prepared according to the general procedure described for TMC-95A and TMC-95B described below. As depicted in Scheme 1, the 17-membered ring is accomplished by macrolactamization. With the ring in place, addition of the unusual, (Z)-1-propenylamide and 3-methyl-2oxopentanoate side chains at C-8 and C-14 respectively (or other analogous side chains for the synthesis of novel derivatives) is conducted. The installation of hydroxyl groups at C-6 and C-7 can be conducted prior to, or after, macrocyclization (Scheme 1). Additonally, a Suzuki reaction (For a review of Suzuki coupling reactions, see: N. Miyaura, A. Suzuki, *Chem. Rev.* 1995, 95, 2457-2483) allows for the joining 6 + 7, and can be employed to reach macrolactamization precursors en route to 5.
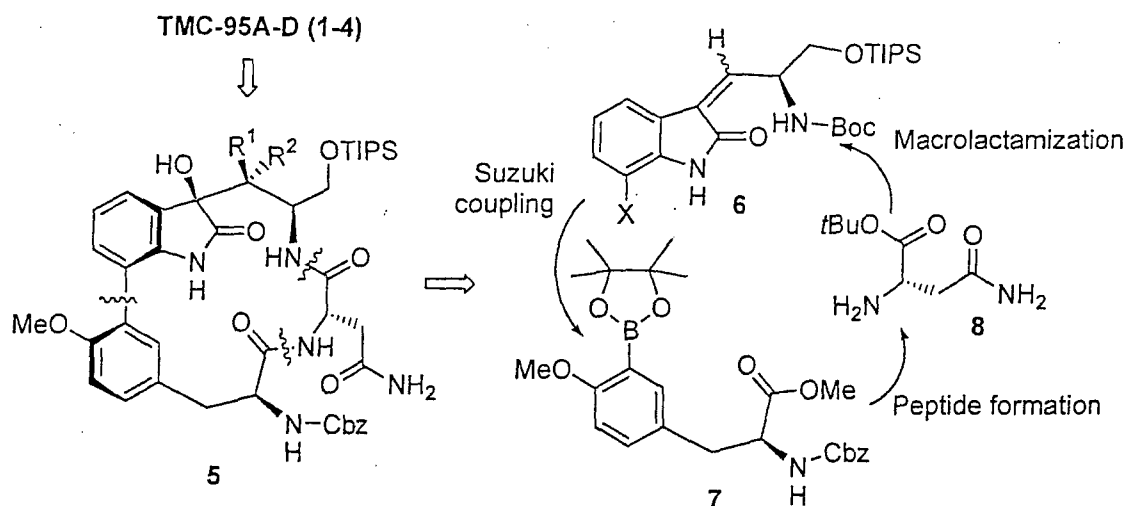
Scheme 1: Retrosynthetic plan.

TIPS = triisoproylsilyl, Cbz = benzoxycarbonyl, Boc = *tert*-butoxycarbonyl, X = Br or I.

The synthesis of oxindole **6a** was first attempted using a proposed palladium mediated intramolecular Heck reaction of substituted *N*-acryl-2,6-dibromoaniline **12**. Thus, methyl ester **9** derived from D-serine (A. McKillop, R. K. Taylor, R. J. Watson, N. Lewis, *Synthesis* **1994**,

31-33) was reduced with DIBAL. This was followed by treatment with methyl (triphenylphosphoranylidine)acetate to provide **10** in 88% yield over two steps. Methyl ester **10** was then converted to the corresponding acid chloride **11** under neutral conditions in a three-step procedure as shown (A. Wissner, C. V. Grudzinskas, *J. Org. Chem.* **1978**, *43*, 3972-3974).

Acylation of 2,6-dibromoaniline with **11** afforded **12** in moderate yield. Attempted cyclization of dibromide **12** to 7-bromooxindole **6a** provided the desired compounds in low yields (5-15%) (Scheme 2).
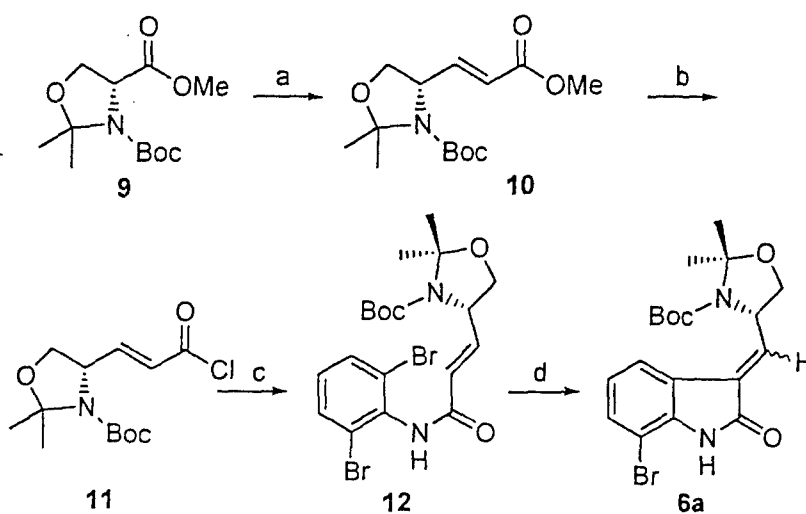
Scheme 2: Synthesis of 7-bromooxindole **6a**. (a) i. DIBAL/toluene, -78 °C, 1 h; ii. methyl (triphenylphosphoranylidine)acetate, CH$_2$Cl$_2$, rt, 88% (2 steps); (b) i. LiOH, THF/MeOH/H$_2$O; ii. TBS-Cl, Et$_3$N/DMAP; iii. (COCl)$_2$, DMF (cat.); (c) 2,6-dibromoanaline, NaH, DMF/THF, 75°C, 1.5 h, 44%; (d) [Pd(PPh$_3$)$_4$] or Pd(OAC)$_2$, 5-15%. DIBAL = diisobutylaluminum hydride, TBS = *tert*-butyl-dimethylsilyl, DMAP = 4-dimethylaminopyridine.

Accordingly, an alternative approach was investigated (Scheme 3). Protection of *N*-Boc-D-serine as shown, was followed by reduction of the methyl ester to provide aldehyde **13** in high yield. The required 7-iodoisatin **16** was obtained in good yield in two steps from 2-iodoaniline **14** *via* isonitrosoacetanilide **15** ((a) C. S. Marvel, G. S. Hiers, *Org. Syn.* **1941**, *Coll. Vol. I*, 327-330. (b) M. S. Newman, M. W. Logue, *J. Org. Chem.* **1971**, *36*, 1398-1401; (c) V. Lisowski, M. Robba, S. Rault, *J. Org. Chem.* **2000**, *65*, 4193-4194).

Upon heating this compound (**16**) with hydrazine hydrate (C. Crestini, R. Saladino, *Syn.*

Commun. 1994, *24*, 2835-2841) for 1 h at 125°C, and subsequent treatment with HCl (6N) at 60 °C for 2 h, 7-iodooxindole **17** was obtained in excellent yield. Condensation of 7 iodooxindole **17** and aldehyde **13** was then carried out under several conditions as summarized in Table 1. As seen, serviceable yields (74-76%) with little or no racemization (up to 96% ee) could be achieved in reaching **6b** (entries 3 and 4) (Similar results have been recently reported: D. Ma and Q. Wu, *Tetrahedron lett.* 2000, *41*, 9089-9093). The structure of the major component of **6b** was confirmed by NOE experiments (see Figure 2) to be *E*-isomer.
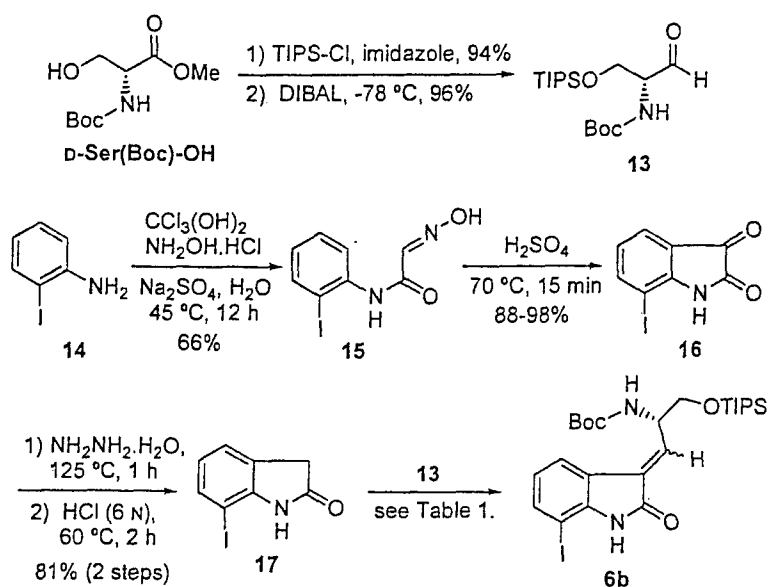
Scheme 3: Synthesis of 7-iodooxindole **6b**.

Table 1. Condensation of oxindole **17** with aldehyde **13**.

Entry	Conditions	Yield (%)[a]	E/Z[b]	ee (%)[c]
1	piperidine (cat.), MeOH or EtOH, 65 °C, 2-3 h	44-50	2.0/1	0
2	piperidine (cat.), THF, rt, 17-44 h	55	1.7/1	~10
3	i) LDA (2.1 equiv.), THF, -78 °C, 1 h; ii) TEA (2.5 equiv.), MsCl (1.2 equiv.), CH₂Cl₂, -60 to -30 °C, 2 h	76	1.3/1	92
4	i) LDA (2.0 equiv.), THF, -78 °C, 1.5 h;			

ii) TEA (3 equiv.), MsCl (1.5 equiv.), CH₂Cl₂, - 70 to -50 ˚C, 1.5 h	74	1.2/1	96

[a]Isolated yield; [b]Determined by ¹H NMR, two isomers are separable; [c]Determined by chiral HPLC.


6b-E   **6b-Z**
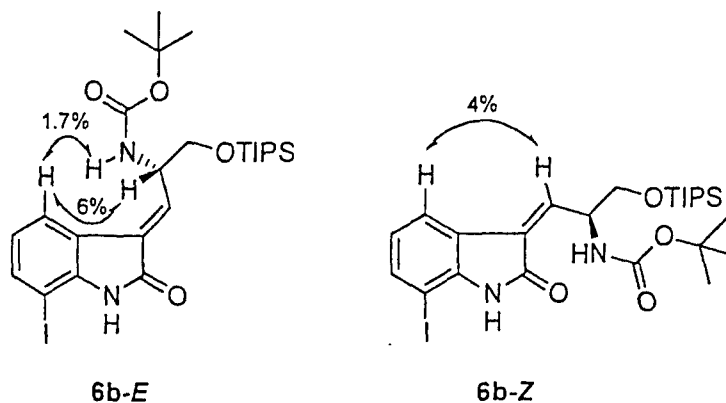
Figure 2: NOE experiments on compounds **6b-E** and **6b-Z** (CDCl₃, 400 MHz).

The aryl borate **7**, required for Suzuki cross-coupling, was synthesized starting from L-tyrosine as shown in Scheme 4. Methyl ester formation with MeOH/SOCl₂ followed by protection of the amino group as a benzylcarbamate, afforded phenol **18** in 96% yield over two steps. *O*-methylation of **18** with Me₂SO₄ in the presence of LiOH·H₂O in dry THF (A. Basak, M.K. Nayak, A. K. Chakraboti, *Tetrahedron lett.* **1998**, *39*, 4883-4886) provided **19** in 86% yield. Selective iodination of **19** at the 3-position *ortho* to the methoxy group was achieved in high yield with I₂/AgSO₄ (W.W. Sy, *Tetrahedron Lett.* **1993**, *34*, 6223-6224) in methanol. Iodide **20** was then converted into aryl borate **7** in 95% yield under Miyaura's conditions using bis(pinacolato)-diboron, [PdCl₂(dppf)]·CH₂Cl₂, and KOAc in DMSO for 13 h at 80 ˚C ((a) T. Ishiyama, M. Murata, N. Miyaura, *J. Org. Chem.* **1995**, *60*, 7508-7510; (b) A. M. Elder, D. H. Rich, *Org. Lett.* **1999**, *1*, 1443-1446).
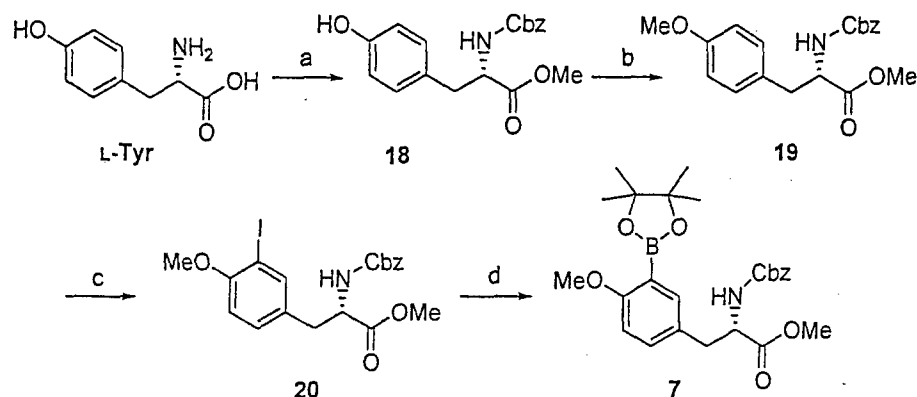
Scheme 4: Synthesis of aryl borate **7**. (a) i. MeOH/SOCl₂; ii. Cbz-Cl, K₂CO₃, H₂O/acetone, 96% (2 steps); (b) LiOH, Me₂SO₄, 86%; (c) I₂, Ag₂SO₄, MeOH, rt, 1.5 h; 93%; (d) bis(pinacolato)-diboron, [PdCl₂(dppf)]·CH₂Cl₂, KOAc, DMSO, 80 °C, 13 h, 95%. dppf = bis(diphenylphosphino)ferrocene.

The appropriate components, 7-iodooxindole **6b** and aryl borate **7**, were indeed joined by a Suzuki coupling (For a review of Suzuki coupling reactions, see: N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457-2483; also, see: T. Ishiyama, M. Murata, N. Miyaura. *J. Org. Chem.* **1995**, *60*, 7508-7510). After an extensive survey of reaction conditions (catalyst loading, temperature and reaction time, equivalents of borate and base) we identified a regimen that afforded coupling products **21** in 72% yield with an *E/Z* ratio of 2/1. The two isomers were separable by silica gel chromatography, and the *E/Z* stereochemistry of each was determined by NOE experiments similar to those conducted on **6b**. No epimerization of the allylic amine position was observed in this reaction. However, it should be noted that *E/Z*-isomerization apparently did occur during the Suzuki process. An *E/Z* ratio of ca. 2/1 was consistently obtained starting with *E*-isomer **6bE**, *Z*-isomer **6bZ**, or a mixture of the two isomers. Conversion of *Z*-isomer **21Z** to *E*-isomer **21E** can be affected by heating **21Z** in DME at 80 °C for 1 day in the presence of catalytic amount of iodine (Scheme 5).
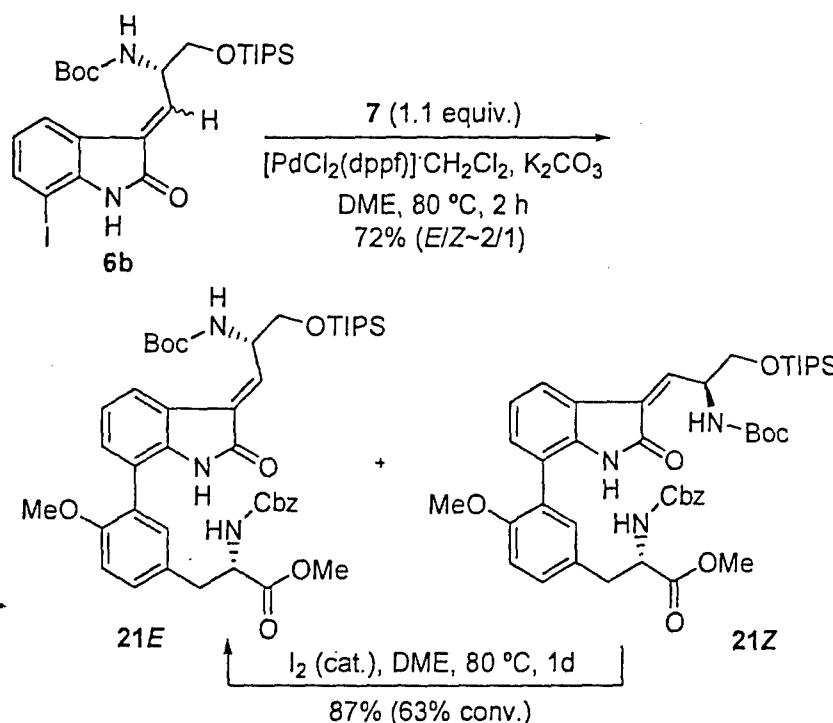
Scheme 5: Biaryl linkage formation by Suzuki coupling. DME = 1,2-dimethoxyethane.

L-asparagine was then appended to the seco framework. Thus, hydrolysis of the α-methyl ester of **21**, and conversion of the derived acid to its *N*-hydroxysuccinimade ester paved the way for amide formation with L-Asn hydrate (see compound **22**). Exposure of **22** to the action of HCl (4N), followed by cyclization of the resulting amino acid with pentafluorophenyl diphenylphosphonate (FDPP) and DIEA in DMF, or with EDC/HOAT in various solvents (DMF, CH₂Cl₂, MeCN), however, did not provide the cyclized product. Without wishing to be bound by any particular theory the rigidity of the *exo* double bond at 3-position of oxindole ring probably tilts the amino group away from the asparagine moiety, thereby preventing cyclization. Accordingly, we decided to accomplish dihydroxylation prior to cyclization. Saponification of the methyl ester **21E** followed by coupling with L-asparagine *tert*-butyl ester (**8**) as before provided **23a** in 70% yield over two steps. Treatment of **23a** with HF/pyridine afforded free alcohol **23b** (Scheme 6). Dihydroxylation on **23a** using OsO₄/NMO in the presence of (DHQD)₂-PHAL (K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K.-S. Jeong, H.-L. Kwong, K. Morikawa, Z.-M. Wang, D. Xu, X.-L. Zhang, *J. Org. Chem.* 1992, *57*, 2768-71) at room temperature afforded the diols **24** in 84% yield (*S/R*~1/1.8),

(The *R* configuration at C6 of **24R** was assigned by converting **24R** to its primary-secondary diol acetonide, whereupon the coupling constant between H7 and H8 is 0 Hz, indicating their *syn*-relationship) along with a small amount of isatin **25** (<5%). Dihydroxylation of **23b** in the presence of (DHQ)$_2$-PHAL (K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K.-S. Jeong, H.-L. Kwong, K. Morikawa, Z.-M. Wang, D. Xu, X.-L. Zhang, *J. Org. Chem.* 1992, *57*, 2768-71) followed by selective reprotection of the primary hydroxyl group gave similar results (81%, *S/R*~1/1.4, Scheme 6).
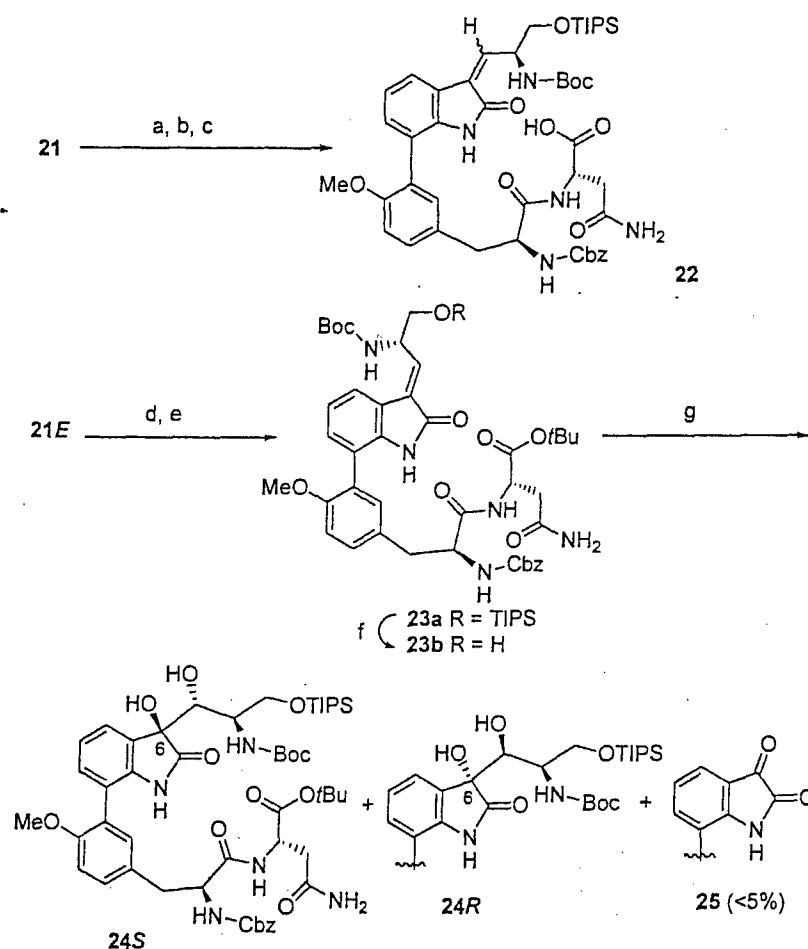
Scheme 6: Synthesis of diols **24**. a) LiOH, THF/MeOH/H$_2$O; b) hydroxysuccinimade, DCC, THF, 55% (2 steps); c) L-Asn.H$_2$O, Et$_3$N,THF/H$_2$O, rt, 4 h, 70%; d) LiOH, THF/H$_2$O, 0 °C, 1.5 h; e) H-Asn-O*t*Bu (8), EDC/HOAT, THF, rt, 2 h, 70% (2 steps); f) HF/Py, 84%; g) a: OsO$_4$/NMO, (DHQD)$_2$-PHAL, *t*BuOH/H$_2$O, rt, 1h, 84% (*S/R*~1/1.8); b: OsO$_4$/NMO, (DHQ)$_2$-PHAL, *t*BuOH/H$_2$O, rt, 4h; TIPS-Cl, imidazole/DMAP, 5 h, 81% (*S/R*~1/1.4). DCC = 1,3-dicyclohexylcarbodiimide, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOAT = 1-hydroxy-7-azabenzotriazole, Asn = asparagine, NMO = 4-

methylmorpholine *N*-oxide, (DHQD)$_2$-PHAL = 1,4-bis(9-*O*-dihydroquinidine)phthalazine, (DHQ)$_2$-PHAL = 1,4-bis(9-*O*-dihydroquinine)phthalazine.

Treatment of **24S** with TFA in CH$_2$Cl$_2$ resulted in concurrent removal of the Boc protecting group and hydrolysis of the *tert*-butyl ester. The crude amino acid was then submitted to macrolactamization using EDC/HOAT under highly dilute conditions (4x10^{-3} M) in CH$_2$Cl$_2$/DMF (4/1). Cyclization progressed smoothly, providing the desired product **5** (The atropisomer shown for **5** follows from the C6 stereochemistry) in 55% yield over two steps (Scheme 7). The large coupling constant (10.4 Hz) observed for H7-H8 in **5**, is similar to those observed in TMC-95A and B (**1** and **2**) (J. Kohno, Y. Koguchi, M. Nishio, K. Nakao, M. Kuroda, R. Shimizu, T. Ohniki, S. Komatsubara *J. Org. Chem.* **2000**, *65*, 990-995), further confirmed the configurational assignments at C6 and C7. It is interesting to note that treatment of *R*-isomer (**24R**) under the same reaction conditions did not afford any cyclization product.
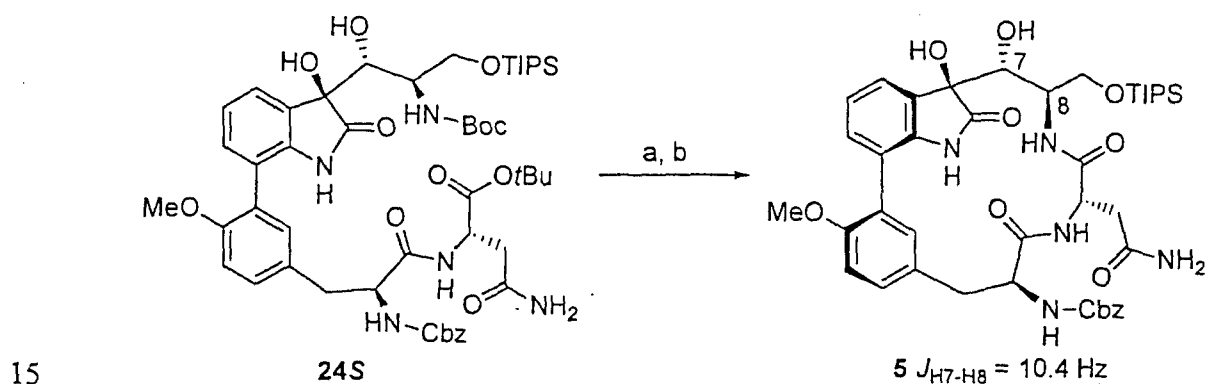
Scheme 7: Macrolactamization of **24S**. a) TFA/CH$_2$Cl$_2$ (4/1), rt, 2h; b) EDC, HOAT, DIEA, CH$_2$Cl$_2$/DMF (4/1, 4 mM), rt, 20 h, 55% (2 steps). DIEA = *N,N*-diisopropylethyl amine.

As described above, the advanced structure (**2**) depicted in Scheme 8 below was synthesized. The attainment of this subgoal still left unaddressed several issues for the total syntheses of **1a** and **1b**. These issues include provision to allow exposure of a free phenolic hydroxyl at C19 and a homopyruvoyl acyl group at N33 (see structure **2**, Scheme 8). Moreover, the oxidation level at C25 must be upgraded from an alcohol found in **2** to the carboxyl state required to reach **1** (vide infra). Additionally, in the synthesis of **2**, the facial

sense of cis hydroxylation at C6 and C7 was virtually stereorandom (S. Lin, S. J. Danishefsky *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 1967-1970). Finally, cis propenamide linkage still needed to be fashioned.


TMC-95	R¹	R²	R³	R⁴
A (1a)	H	OH	CH₃	H
B (1b)	H	OH	H	CH₃
C (1c)	OH	H	CH₃	H
D (1d)	OH	H	H	CH₃
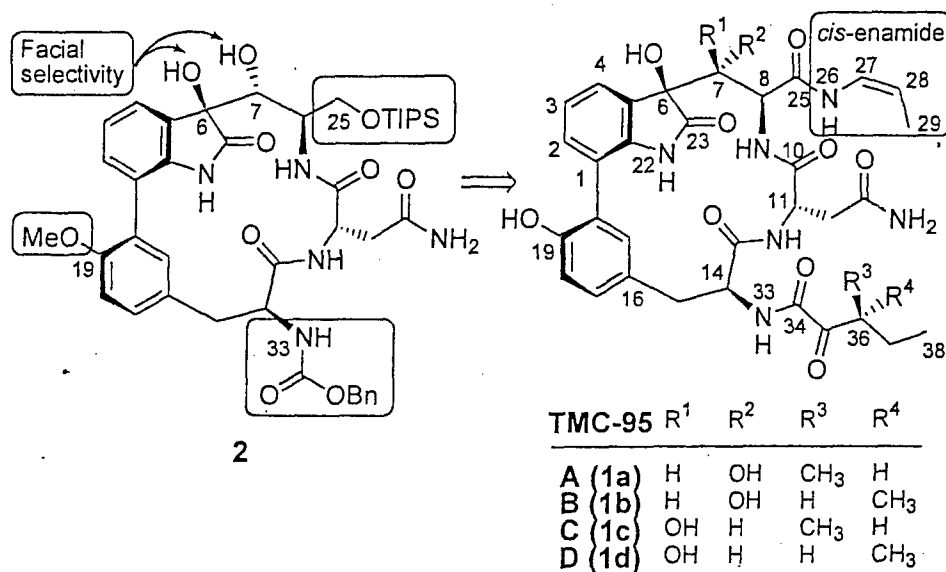
Scheme 8: Structures of TMC-95-A–D (**1a – d**) and **2**. TIPS = triisoproylsilyl, Bn = benzyl.

Examination of the literature reveals several methods that might in principle be brought to bear on the enamide problem (a) J. K. Stille, Y. Becker, *J. Org. Chem.* **1980**, *45*, 2139-2145; b) P. Ribereau, M. Delamare, S. Celanire, G. Queguiner, *Terahedron Lett.* **2001**, *42*, 3571-73; c) K. Kuramochi, H. Watanabe, T. Kitahara, *Synlett* **2000**, *3*, 397-399; d) R. Chen, J. A. Porco, Jr. *Org. Lett.* **2000**, *2*, 1333-1336).

However, the applicability of these references to the culminating phase *en route* to the active TMC compounds prompted no small concern. Compounds **1** have a rich diversity of functionality, particularly in the extended "pyruvoyl"-like (C34 → C38) and dihydroxyindolinone (positions 22, 23, 6, 7, 8 and 28) regions. Anticipation of potential vulnerability in these sectors, not to speak of the *cis*-enamide itself (C26-C29), prompted us to explore a new modality for reaching such a substructure appropriate for molecules laden with multiple sites of potential instability. Referring to Scheme 9, we wondered whether a substance of the type **4** might, upon appropriate thermolysis or catalysis, undergo concurrent

ene- and silatropic-like bond reorganizations leading to **5** (Scheme 9). If this hypothesis were to be fruitful, the *cis* character of the enamide would be virtually assured at the kinetic level. Moreover, it seemed at least possible that the intermediate silyl imidate linkage in **5** could be cleaved, with retrieval of general substructure **6**.

5
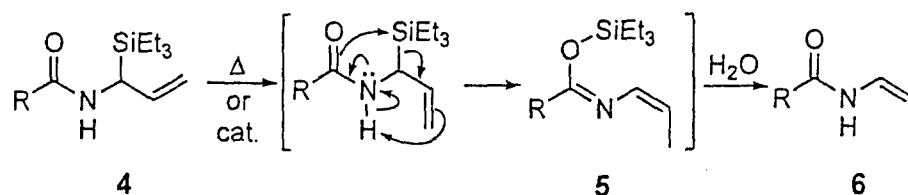
Scheme 9: Formation of *cis*-propenamides **6** via rearrangement-hydrolysis of α-silylallyl amides **4** (R = aromatic, alkenyl, and alkyl groups).

10

In the event, as depicted in Scheme 10, a range of probe substrates **4**, was synthesized by appropriate acylation of the known of amine **3** (This known amine (S.-F. Chen, E. Ho, P. S. Mariano, *Tetrahedron* **1988**, *44*, 7013-7026) was synthesized from allyl alcohol using an improved procedure via a one-pot TES ether formation, retro-Brook rearrangement, and

15 mesylation, followed by a displacement of mesylate with ammonia. Acylation in cases (a) – (d) was accomplished via coupling of amine **3** with the acid chlorides, while in case (e) a EDCI-mediated coupling with protected amino acid with amine **3** was involved. As seen in entries a-c, thermolysis of these compounds at ca. 110 °C for the time periods indicated, gave rise to silyl imidates **5a** – **c** (observed *via* ^{1}H NMR analysis) (The formation of silyl imidates **5**

20 was clearly observed *via* ^{1}H NMR when these reactions were carried out in deuterated solvents). Aqueous hydrolysis of these compounds afforded enamides **6a** – **c** (Table 2). The reaction was applicable to substrate **4d**, though a longer thermolysis time was required for its conversion to **5d**. *Most significantly, the thermolysis – hydrolysis sequence proved extendable to the aminoacyl substrate 4e, leading to 5e and thence to 6e.* It was of great interest to

25 determine whether this new method would find application at a very late stage of our projected total synthesis.

**Table 2.** Thermolysis-hydrolysis of α-silylallyl amides 4.
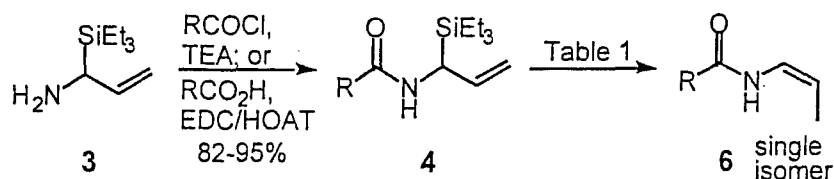
Entry	R	Conditions	Yield (%)
a		1) toluene, 110 °C, 10 h; 2) H_2O	81
b		1) toluene, 110 °C, 20 h; 2) H_2O	73
c		1) toluene, 110 °C, 27 h; 2) H_2O	67
d		1) toluene, 110 °C, 3 d; 2) H_2O	72
e		1) *o*-xylene, 135 °C, 4 d; 2) H_2O	52

As depicted in Scheme 10, the starting material for our refashioned synthesis was the previously described iodooxindole **7** described above. Crossed aldol condensation of **7** with the Garner aldehyde **8** (a) P. Garner, J. M. Park, *J. Org. Chem.* **1987**, *52*, 2361-2364; b) A. McKillop, R. K. Taylor, R. J. Watson, N. Lewis, *Synthesis* **1994**, 31-33. Garner's aldehyde was employed since it was found that an *N,O*-acetonide was crucial for the stereoselective installation of diol functionality at C6-C7 position followed by β-elimination of the derived mesylate afforded a 1:1.3 mixture of α,β-unsaturated lactams **9**(*Z*) : **9**(*E*) (see, S. Lin, S.J. Danishefsky *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 1967-1970; D. Ma, Q. Wu *Tetrahedron Lett.* **2001**, *42*, 5379-5281). Fortunately, the former isomer could be converted to the latter one *via* iodine-mediated isomerization as shown (Scheme 10). In a parallel sequence, L-tyrosine

(10) was converted to 11 in three steps as shown (To address the issues raised by problematic deprotection of the phenolic methyl group at a later stage (i.e., compound 2, Scheme 8), a benzyl group, instead of a methyl group, was used for the protection of phenol, cf. reference 4a). A high yielding ortho iodination[4a] of 11 led to 12 and thence, following palladium mediated borylation ((a) T. Ishiyama, M. Murata, N. Miyaura, *J. Org. Chem.* **1995**, *60*, 7508-7510; b) A. M. Elder, D. H. Rich, *Org. Lett.* **1999**, *1*, 1443-1446), to 13. Suzuki type coupling (For a review of Suzuki coupling reactions, see: N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457-2483) of 13 with 9 afforded compound 14 (75% yield). We hoped that the biaryl domain thus presented, would be serviceable in the context of our projected total synthesis (*vide infra*).
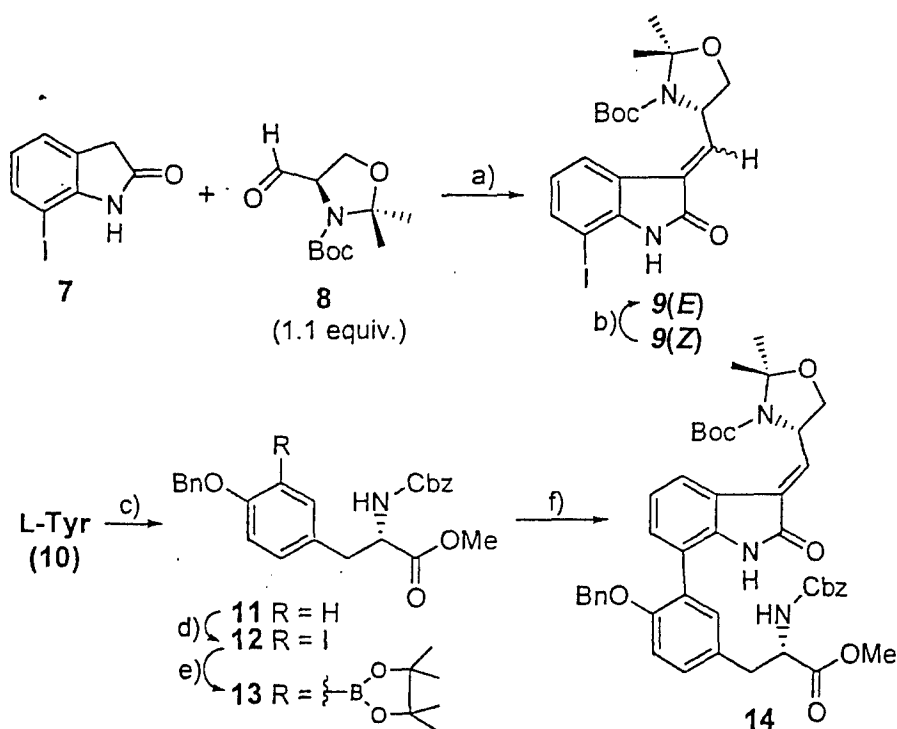
Scheme 10: Synthesis of biaryl compound 14. key: a) LDA (2.0 equiv.), THF, -78 °C, 1.5 h; TEA, MsCl, CH₂Cl₂, -70 to -50 °C, 1.5 h; 81% (E/Z= 1.3/1); b) I₂ (cat.), benzene, 80 °C, 26 h; DMP/PPTS, toluene, 65 °C, 5h; 85% (60% con.); c) 1) MeOH/SOCl₂; 2) Cbz-Cl/K₂CO₃; 3) BnBr,Cs₂CO₃, acetone, reflux; 88% (3 steps); d) Ag₂SO₄/I₂, MeOH, rt, 1 h; 99%; e) pinacolatodiborane, [PdCl₂(dppf)]·CH₂Cl₂, KOAc, DMSO, 80 °C, 10 h, 91%; f) 9(E), [PdCl₂(dppf)]·CH₂Cl₂, K₂CO₃, DME, 80 °C, 2 h; 75%. Boc = *tert*-butoxycarbonyl, Cbz = benzoxycarbonyl, DMP = 2,2-dimethoxypropane, PPTS = pyridinium *p*-toluenesulfonate, dppf = bis(diphenylphosphino)ferrocene.

Hydrolysis of the methyl ester function of **14** led to the corresponding carboxylic acid, which, following acylation of the basic nitrogen of asparagine derivative **15**, afforded **16**. The hydroxyl groups were introduced at carbons 6 and 7 as shown (Scheme 11). Indeed, the presence of the Garner *N,O*-acetonide served to direct, preferentially, the oxidizing agent to the *Re* face (C6) of **16**, thus affording **17** in a 5:1 ratio relative to its 6*R*, 7*S* stereoisomer (not shown).
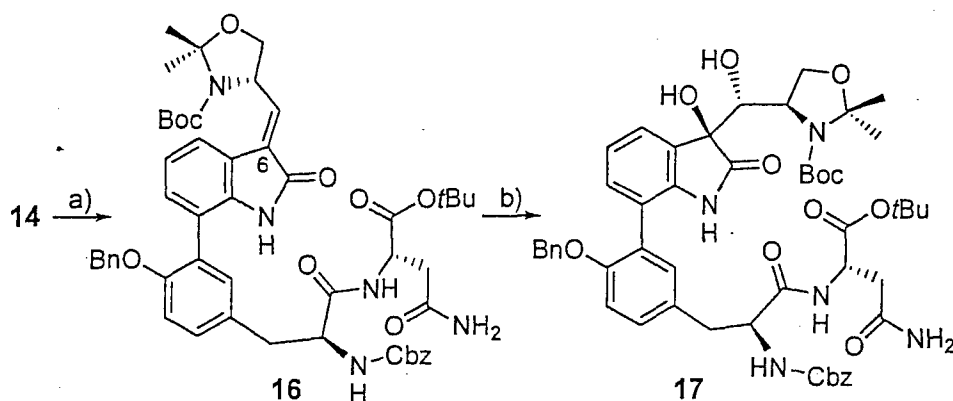
Scheme 11: Synthesis of diol 17. Key: a) 1) LiOH, THF/H₂O, 0 °C, 1.5 h; 2) H-Asn-O*t*Bu (15), EDC/HOAT, THF, rt, 2 h; 85% (2 steps); b) OsO₄/NMO, (DHQD)₂-PHAL, *t*BuOH/H₂O, rt, 12 h; 88% (*S/R* = 5/1). Asn = asparagine, EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, HOAT = 1-hydroxy-7-azabenzotriazole, NMO = 4-methylmorpholine *N*-oxide, (DHQD)₂-PHAL = 1,4-bis(9-*O*-dihydroquinidine)phthalazine.

The timing and manner in which the various heteroatom-centered functional groups were exposed and protected proved to be important. As depicted in Scheme 12, deprotection of the *N,O*-isopropylideneacetal linkage of **17** afforded *N*-Boc triol **18**. The primary alcohol at position 25 was protected as its TIPS derivative (see **19**). Cleavage of the *t*-butyl group generated a carboxylic acid at C10, thereby setting the stage for macrolactamization (see compound **20**) (The atropisomer shown for **20** follows from the C6 stereochemistry (see reference 2b). In the next step, the benzyl groups protecting the C19 phenol and the nitrogen comprising position 33 were concurrently cleaved by hydrogenolysis. Acylation of the basic nitrogen (position 33) with racemic **21** (3-Methyl-2-oxopentanoic acid (**21**) was obtained from

its commercially available sodium salt. There would be no purpose in coupling with enantiomerically defined acid **21** because the C36 stereocenter epimerizes rapidly throughout the series) afforded **22a** and **22b** as a 1:1 mixture. Both compounds were advanced concurrently. The TIPS protecting group was cleaved from the primary alcohol. The four

5   hydroxyl groups (positions 6, 7, 19 and 25) were protected as the tetra-TES derivatives (see **23a** and **23b**). In a key step of the synthesis, reaction of these compounds with Jones Reagent (a) K. Bowden, I. M. Heibron, E. R. H. Jones, B. C. L. Weedon, *J. Chem. Soc.* **1946**, 39; b) R. A.Pilli, M. M. Victor, *Tetrahedron Lett.* **1998**, *39*, 4421-4424) led to specific oxidation at the primary center (position 25) to afford acids **24a,b**. In addition, partial deprotection occurred at

10 the C19 ether linkage resulting in the formation of **25a,b**. These four component mixture, following condensation with amine **3** as indicated, led to the amide silyl ethers **26a,b** as well as amide phenols **27a,b** (**26a,b** : **27a,b** ~ 1/1.5).
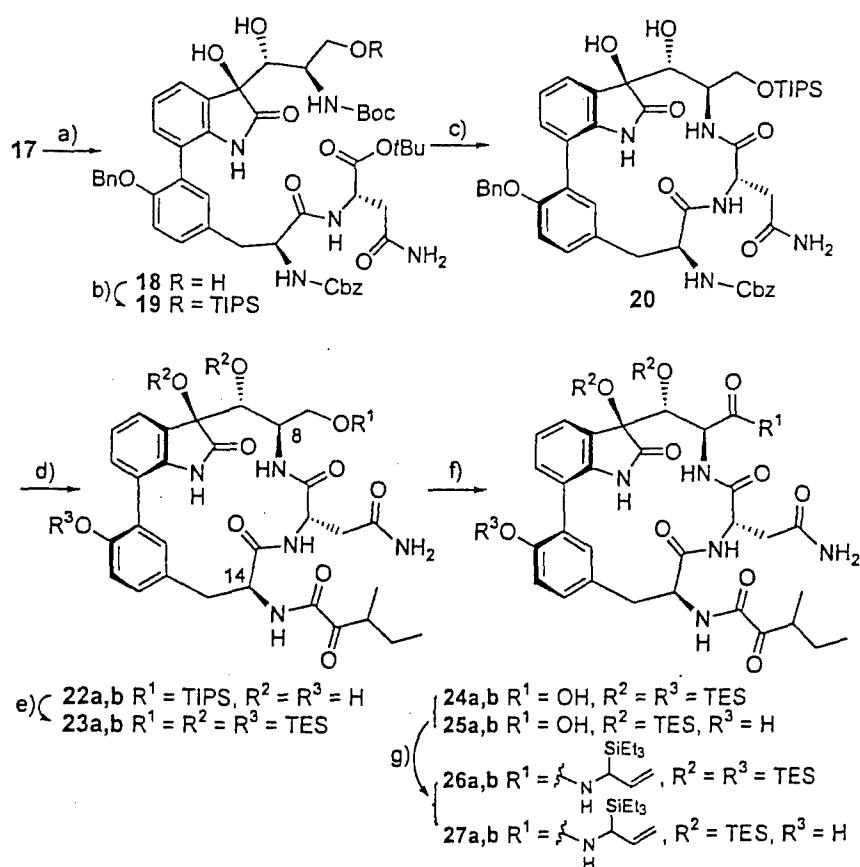
15 **Scheme 12:** Synthesis of α-silylallyl amides **26a,b** and **27a,b**. Key: a) PPTS/MeOH, reflux, 2 h; b) TIPS-Cl, imidazole/DMAP, CH₂Cl₂, rt, 5 h; 88% (2 steps); c) 1) TFA/CH₂Cl₂ (4/1), rt,

2h; 2) EDC/HOAT/DIEA, CH_2Cl_2/DMF(2 mM), rt, 24 h; 52% (2 steps); d) 1) Pd/C, H_2, EtOH, rt, 19 h; 2) (±)-3-methyl-2-oxopentanoic acid (21), EDC/HOAT, CH_2Cl_2/DMF, rt, 2 h; 85% (2 steps); e) 1) HF/Py; 2) TES-OTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 15 h; 3) $NaHCO_3$; 4) Citric acid, EtOAc/H_2O; 73% (from 22); f) Jones reagent, acetone, 0 °C, 2 h; g) 3, EDC/HOAT, CH_2Cl_2/DMF, rt, 13 h; 45% (2steps). DMAP = 4-dimethylaminopyridine, DIEA = *N,N*-diisopropylethyl amine.

Construction of the (*Z*)-1-propenylamide was now achieved by thermolytically driven rearrangement of the α-silylallyl amides corresponding to 4 (Scheme 13). The rearrangement of the complex mixture (It was not feasible or necessary to separate at this stage. Rather the eight component mixture was advanced as shown, and the separation was achieved at the stage of the two component mixture 1a,b), in anhydrous *o*-xylene at 140 °C, provided (*Z*)-1-propenylamides 28a,b and 29a,b. The crude mixture of these compounds was globally deprotected with pyridine-buffered HF/pyridine, to afford a mixture of our total synthesis goals – TMC-95A and B (1a and 1b; 1/1). This mixture was separated by HPLC using a reverse phase column (HPLC conditions are as follows: Column: YMC-pack ODS-AM, 150 X 10 mm; eluant: 25% MeCN in water; flow rate: 2.5 mL/min; T_{R1a} = 37.3 min, T_{R1b} = 34.3 min. The synthetic materials were identical to natural TMC-95A & B (Rf, NMR, EIMS, and HPLC) and we thank Dr. Jun Kohno, Tanabe Seiyaku Co., Japan, for generously providing us with the HPLC conditions and the samples of TMC-95A & B for comparison) to provide the individual compounds 1a and 1b. These compounds were characterized by their high field NMR spectra in comparison with those of authentic samples.
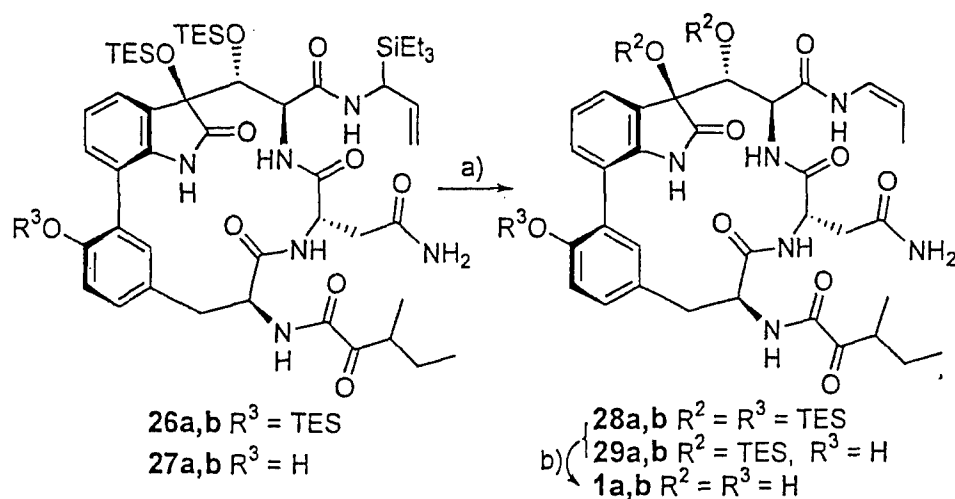
Scheme 13: Synthesis of TMC-95A and B (**1a,b**). Key: a) 1) *o*-xylene, 140 °C, 3 d; 2) H₂O; b) HF/Py, THF/Py; then Me₃Si-OMe; 49% (2 steps).

In summary, the total syntheses of TMC-95A & B have been achieved. The program featured a sequential assembly of oxindole **7**, Garner's aldehyde (**8**), aryl boronate **13**, asparagine derivative **15**, 3-methyl-2-oxopentanoic acid (**21**), and α-silylallyl amine (**3**). Highlights of the synthesis include a venturesome application of the Suzuki biaryl construction (9(*E*) + **13** → **14**), a diastereofacial dihydroxylation reaction taking advantage of the Garner method (**16** → **17**), and a macrolactamization (formation of **20**). We also note new chemistry to accomplish stereo-specific *cis*-propenamide formation (**26** & **27** → **28** & **29**) was inspired by goal system **1**.

Example 2: Generation of analogues of TMC-95A, B, C and D:

It will be appreciated that a variety of synthetic analogues can be generated from the general synthesis and intermediates discussed above. In addition, using synthetic methods available to one of ordinary skill in the art, analogues where the homopyruvoyl acyl moiety can be replaced by a variety of other functional moieties capable of effecting inhibition of proteolysis.

As depicted in the Schemes 14-18 below, a variety of analogues can be synthesized using the novel synthetic methodologies and intermediates described above.

## Schemes for the Synthesis of TMC-95 Derivatives

Employing the method developed in the synthesis of TMC-95A & B (see Example 1), a number of their derivatives (30-33, Schemes 14-16) were accessed readily.

24a,b or 25a,b R³ = TES/H   30a,b
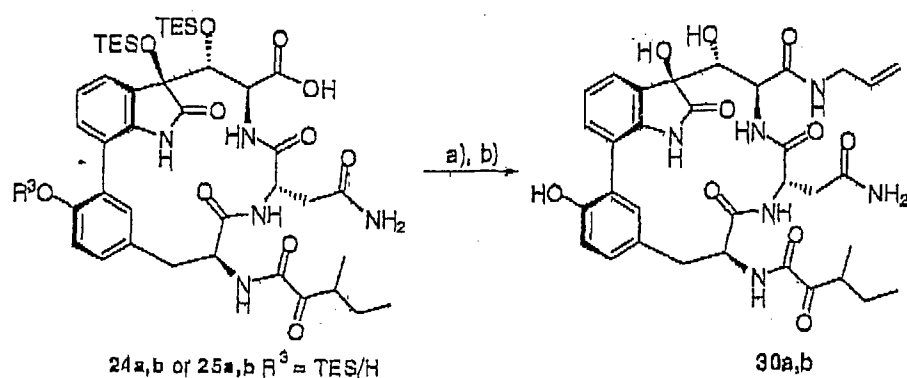
Scheme 14. Synthesis of derivatives 30a,b. a) allyl amine, EDC/HOAT, CH₂Cl₂/DMF; b) HF/Py, THF/Py; then Me₃Si-OMe.

5   31a,b
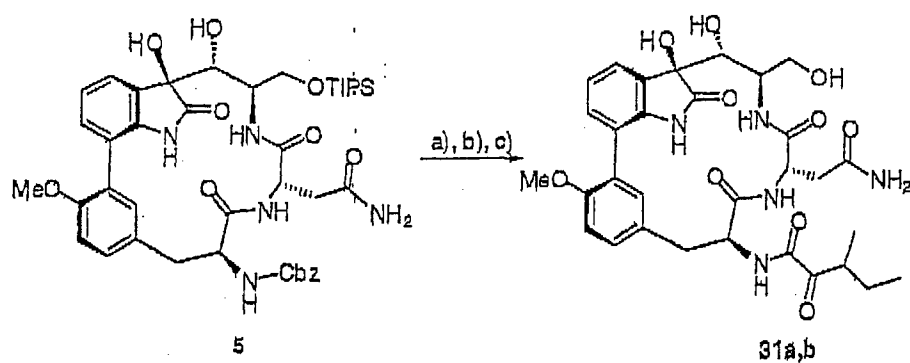
Scheme 15. Synthesis of derivatives 31a,b. a) Pd/C, H₂, EtOH, rt; b) (±)-3-methyl-2-oxopentanoic acid (21), EDC/HOAT, CH₂Cl₂/DMF, rt; c) HF/Py.
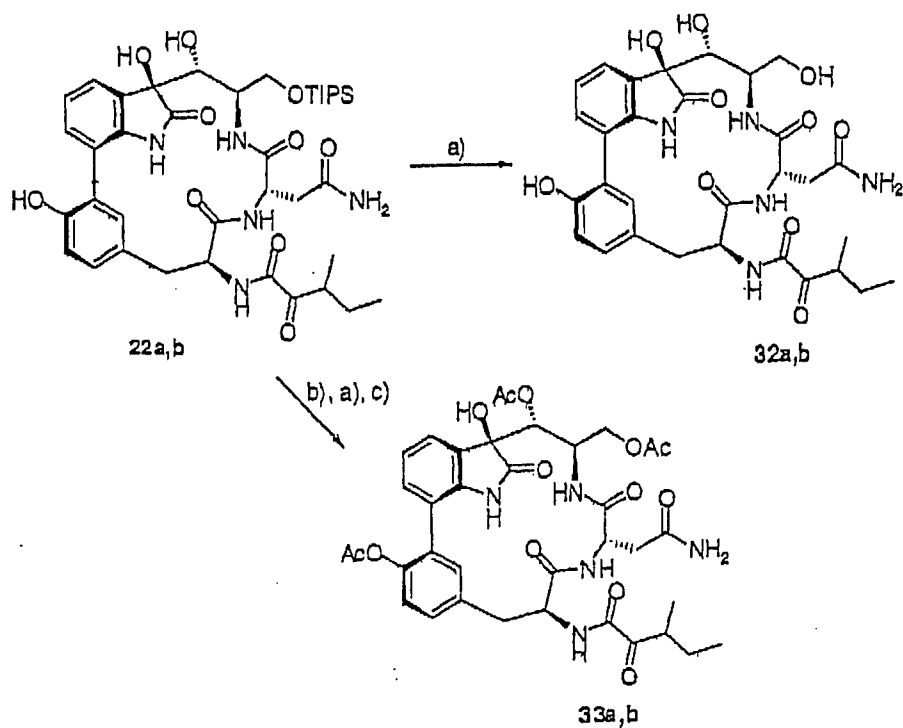
Scheme 16. Synthesis of derivatives 32a,b and 33a,b. a) HF/Py; b) Ac₂O, Py/DMAP, CH₂Cl₂; c) silic gel.

Similarly, derivatives 35a,b can be synthesized readily (Scheme 17).

20

a), b)

34a,b

c), d), e), f)

35a

R^1 = H, Me, Et, isopropyl, 1-propenyl, allyl
R^2 = Me, Et, isopropyl, 1-methylpropyl, isobutyl

35b

R^1 = H, Me, Et, isopropyl, 1-propenyl, allyl
R^2 = Me, Et, isopropyl, tert-butoxy, benzyloxy
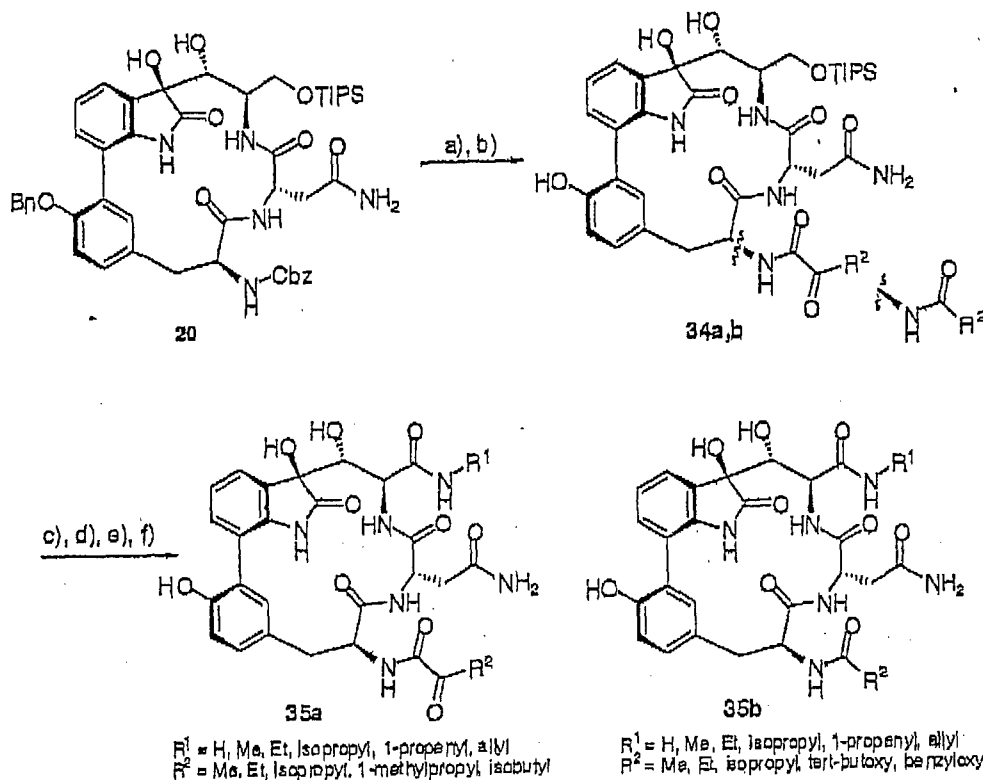
Scheme 17. Synthesis of derivatives **35a,b.** a) Pd/C, H_2, EtOH, rt; b) R^2COCO_2H, EDC/HOAT or R^2COCl, Et_3N/DMAP; c) 1) HF/Py; 2) TES-OTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt; d) Jones reagent, acetone, 0 °C; e) amine, EDC/HOAT; f) HF/Py.

# Structures of TMC95A, B, C, D, and Their Derivatives
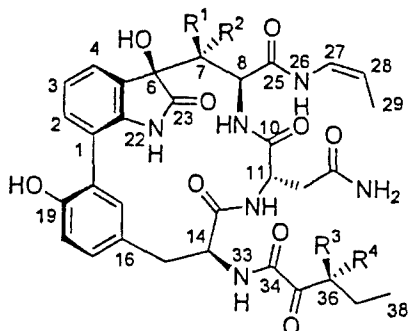
| TMC-95	R^1	R^2	R^3	R^4
A (1a)	H	OH	CH_3	H
B (1b)	H	OH	H	CH_3
C (1c)	OH	H	CH_3	H
D (1d)	OH	H	H	CH_3

Structures of TMC95A, B, C, and D
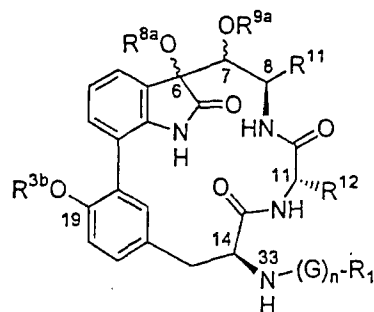
R^{11} = alkyl, alkenyl, aryl, alkoxycarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, etc
R^{12} = alkyl, alkenyl, aryl, etc
$(G)_n$-R_1 = alkyl, alkenyl, aryl, alkoxycarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, 3-substituted pyruvyl, etc
R^{3b} = alkyl, alkenyl, aryl, etc
R^{8a} = alkyl, alkenyl, aryl, alkoxycarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, etc
R^{9a} = alkyl, alkenyl, aryl, alkoxycarbonyl, alkylaminocarbonyl, alkenylaminocarbonyl, etc

Structures of Certain Exemplary Derivatives of TMC95:

R^{11a} = H,

R^{3b} = H, Me;
R^{8a}, R^{9a}, R^{11a} = H, Ac;
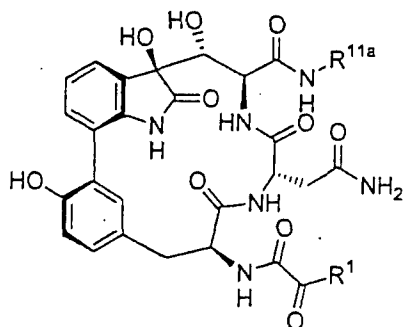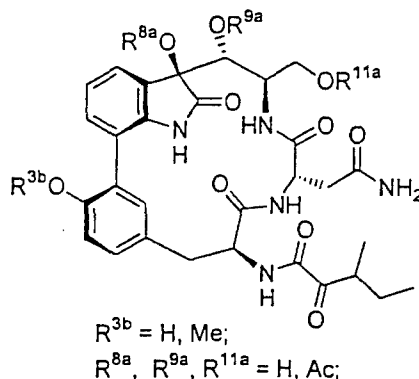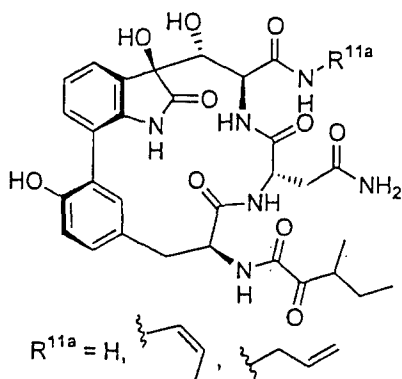
R^{11a}= H, Me, Et, isopropyl, 1-propenyl, allyl
R^1 = Me, Et, isopropyl, 1-methylpropyl, isobutyl
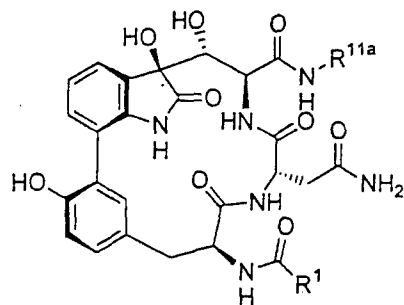
R^{11a}= H, Me, Et, isopropyl, 1-propenyl, allyl
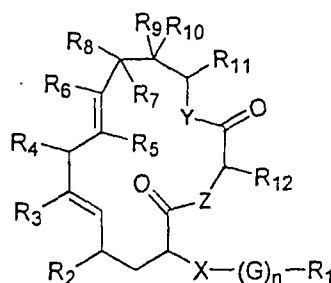R^1 = Me, Et, isopropyl, tert-butoxy, benzyloxy

Scheme 18

2) *Biological Assays:*

It will be appreciated that a variety of assays are available in the art for determining whether the inventive compounds have activity as proteasome inhibitors, as cytotoxic agents, or are useful in the prevention of HIV infection or in the prevention and/or treatment of inflammatory disorders, or other disorders caused by certain regulatory subunits of the proteasome. *See* generally, US Patents 5,780,454; 6,066,730; 6,083,903; 6,265,380; and 6,297,217, the entire contents of which are hereby incorporated by reference.
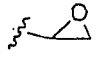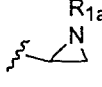
2   *CLAIMS*

3

4   1.   A compound having the structure (**I**):

5

8   (**I**)

9   and pharmaceutically acceptable derivatives thereof;

10   wherein R_1 is hydrogen, $-CH_2Cl$, $-CH_2Ac$, $-C=C-S(O)_2-CH_3$, $B(OR_{1a})_2$, $-(C=O)R_{1a}$, or an

11   aliphatic, heteroaliphatic, aryl or heteroaryl moiety, or   or   ,wherein each

12   occurrence of R_{1a} is hydrogen, an oxygen protecting group, or an aliphatic, heteroaliphatic, aryl,

13   or heteroaryl moiety;

14   each occurrence of G is independently (C=O) or $-CH_2-$;

15   n is 0, 1, 2, or 3;

16   X is NH, O, CH_2, or is absent;

17   Y and Z are each independently NH, N(alkyl), O, S, CH_2, CH(alkyl), or C(alkyl)(alkyl);

18   R_2 and R_3 are each hydrogen, or an aliphatic, heteroaliphatic, aryl, or heteroaryl moiety,

19   or wherein R_2 and R_3 taken together are an aryl, heteroaryl, cycloaliphatic, or

20   heterocycloaliphatic moiety;

21   R_4, R_5, R_6 and R_7 are each hydrogen, or an aliphatic, heteroaliphatic, aryl or heteroaryl

22   moiety, or wherein R_4 and R_6 taken together are an aryl, heteroaryl, cycloaliphatic, or

23   heterocycloaliphatic moiety, and wherein R_5 and R_7, taken together, are an aryl, heteroaryl ,

24   cycloaliphatic or heterocycloaliphatic moiety;

1   R_8, R_9 and R_{10} are each independently hydrogen, protected or unprotected hydroxyl,

2   protected or unprotected thio, protected or unprotected amino, acyl, acyloxy, or an aliphatic,

3   heteroaliphatic, aryl or heteroaryl moiety;

4   R_{11} is hydrogen, an aliphatic, heteroaliphatic, aryl, heteroaryl, alkoxycarbonyl,

5   alkylaminocarbonyl, or alkenylaminocarbonyl moiety or is (alipahtic)OR_{11a}, or

6   (heteroaliphatic)OR_{11a}, wherein R_{11a} is hydrogen, a protecting group, acyl, or an aliphatic,

7   heteroaliphatic, aryl or heteroaryl moiety;

8   R_{12} is hydrogen, an aliphatic, heteroaliphatic, aryl, or heteroaryl moiety, alkoxycarbonyl,

9   alkylaminocarbonyl, alkenylaminocarbonyl, or -(alkyl)(C=O)NH_2,
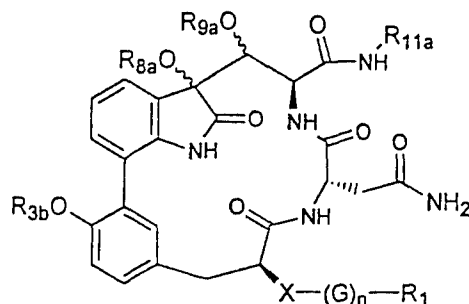
10   whereby each of the foregoing aliphatic and heteroaliphatic moieties is independently

11   substituted or unsubstituted, cyclic or acyclic, linear or branched, and each of the foregoing aryl

12   and heteroaryl moieties is independently substituted or unsubstituted.

13

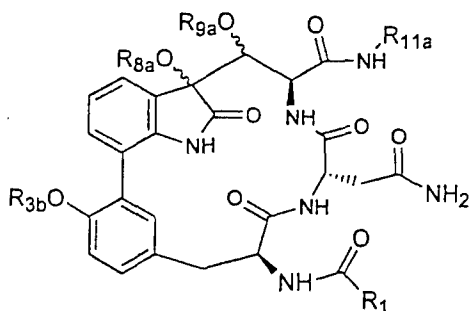14   2.   The compound of claim 1, wherein the compound has the structure:

15
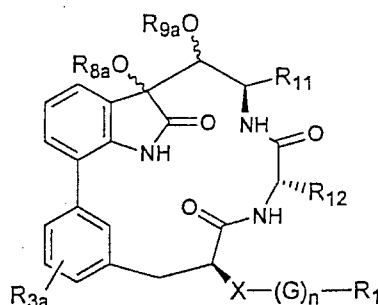
16

17

18   wherein R_{3a} is hydrogen, OR_{3b}, -$N(R_{3b})_2$, SR_{3b}, halogen, or an aliphatic, heteroaliphatic,

19   aryl or heteroaryl moiety, wherein each occurrence of R_{3b} is independently hydrogen, a

20   protecting group, or an aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_{8a} and R_{9a} are

21   each independently hydrogen, acyl, a protecting group, or an aliphatic, heteroaliphatic, aryl or

22   heteroaryl moiety.

23

24   3.   The compound of claim 1, wherein the compound has the structure:
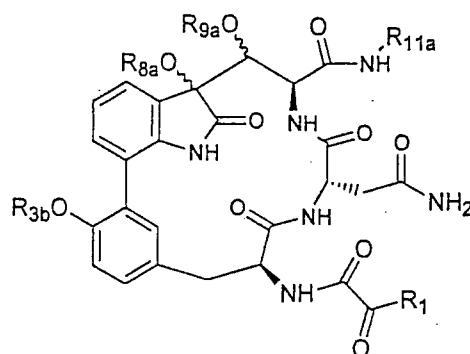
25

1
2

3   wherein R_{3b} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or

4   heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an

5   aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_{11a} is hydrogen, a protecting group, or

6   an aliphatic, heteroaliphatic, aryl or heteroaryl moiety.

7

8   4.   The compound of claim 1, wherein the compound has the structure:

9

10
11

12   wherein R_{3b} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or

13   heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an

14   aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_{11a} is hydrogen, a protecting group, or

15   an aliphatic, heteroaliphatic, aryl or heteroaryl moiety.

16

17   5.   The compound of claim 1, wherein the compound has the structure:

1
2
3   wherein R_{3b} is hydrogen, a protecting group, or an aliphatic, heteroaliphatic, aryl or

4 heteroaryl moiety; R_{8a} and R_{9a} are each independently hydrogen, acyl, a protecting group, or an

5 aliphatic, heteroaliphatic, aryl or heteroaryl moiety; and R_{11a} is hydrogen, a protecting group, or

6 an aliphatic, heteroaliphatic, aryl or heteroaryl moiety.

7

8 6.   The compound of claim 1, with the proviso that the compounds exclude TMC-95A, B, C

9 and D.

10

11 7.   The compound of claim 1, wherein the compound is a purified compound.

12

13 8.   The compound of claim 1, wherein Y and Z are each NH.

14

15 9.   The compound of claim 1, wherein X is NH, n is 2, each occurrence of G is (C=O), and

16 R_1 is lower alkyl or lower alkenyl.

17

18 10.   The compound of claim 1, wherein X is NH, n is 1, G is (C=O) and R_1 is lower alkyl or

19 lower alkoxy.

20

21 11.   The compound of claim 1, wherein R_{11} is (C=O)NHR$_{11a}$ where R_{11a} is lower alkyl or

22 lower alkenyl.

23

24 12.   The compound of claim 1, wherein R_{12} is -CH$_2$(C=O)NH$_2$.

25

1   13.   The compound of claim 1, wherein R_{11} is CH_2OR_{11a}, wherein R_{11a} is hydrogen, a

2   protecting group, acyl, or lower alkyl.

3

4   14.   A pharmaceutical composition comprising a compound of any one of claims 1-13, and

5   pharmaceutically acceptable derivatives thereof; and

6   a pharmaceutically acceptable carrier or diluent, and optionally further comprising an

7   additional therapeutic agent.

8

9   15.   The pharmaceutical composition of claim 14, wherein the compound is present in an

10   amount effective to inhibit the proteasome function in a mammal.

11

12   16.   A method for treating cancer comprising:

13   administering to a subject in need thereof a therapeutically effective amount of a

14   compound of any one of claims 1-13, and pharmaceutically acceptable derivatives thereof; and

15   optionally further administering an additional therapeutic agent.

16.

17   17.   A method for inhibiting proteasome activity comprising:

18   administering to a subject in need thereof an amount of a compound of any one of claims

19   1-13, which amount is effective to inhibit proteasome activity, and pharmaceutically acceptable

20   derivatives thereof, and

21   optionally further administering an additional therapeutic agent.

22

23   18.   A method for treating an inflammatory disorder comprising:

24   administering to a subject in need thereof a therapeutically effective amount of a

25   compound of any one of claims 1-13, and pharmaceutically acceptable derivatives thereof, and

26   optionally further administering an additional therapeutic agent.

ABSTRACT

The present invention provides compounds having formula (I):
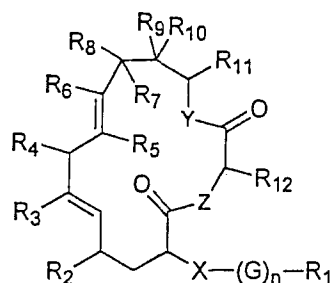
wherein R_1-R_{12}, G, X, Y, Z and n are as described generally and in classes and subclasses herein, and additionally provides pharmaceutical compositions thereof, and methods for the use thereof in the treatment of cancer, inflammatory disorders, and more generally, as inhibitors of proteasome function.

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The Patent and Trademark Office stamping
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APPLICANT: _Danishefsky et al._
TITLE: CYCLIC PEPTIDES, ANALOGUES AND USES
           THEREOF

Comprising: 48 pages of specification; 5 pages of claims; 1 sheet of
abstract; 3 pages of Provisional Application Transmittal

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SCIENCE POLICY: GLOBAL R&D ISSUES CONCERN SCIENTISTS

CHEMICAL
& Engineering News

MARCH 4, 2002

MERGERS &
ACQUISITIONS
Private investors buy
into the chemical industry

Proteasome inhibitors "are absolutely essential in helping to understand the physiological role of proteasomes in cells."

Madeleine M. Joullié, University of Pennsylvania

RESEARCH

ENAMIDE TRANSFORMATION

Novel construction procedure is key to proteasome inhibitor total syntheses

A USEFUL NEW ORGANIC SYNthetic transformation has proven to be an essential element in the first total syntheses of two promising proteasome inhibitors.

The inhibitors are potential drug leads for cancer and other conditions. Clinical trials of a smaller proteasome inhibitor for treatment of cancer are currently being conducted by Millennium Pharmaceuticals.

The new procedure makes it possible to add a cis-enamide group to a structure that already contains delicate functional groups. The technique—devised by chemistry professor Samuel J. Danishefsky of Sloan-Kettering Institute for Cancer Research and Columbia University and postdoctoral colleague Songnian Lin—was developed to facilitate total syntheses of the cyclic peptide natural products TMC-95A

and TMC-95B [*Angew. Chem. Int. Ed.*, **41**, 512 (2002)].

One of the most challenging problems the researchers encountered was the need to add, near the end of the synthesis, a cis-enamide to a substantially complete TMC-95 precursor. Conventional methods for adding such a group could have disrupted delicate groups that had already been assembled in other parts of the synthon.

The researchers solved the problem using an α-silylallyl amine reagent. The amine is attached via an amide bond to a protected TMC-95 precursor. After heating, the amide undergoes bond reorganization, and the imidate product is then hydrolyzed to form the desired cis-enamide moiety.

A German research team that had earlier determined a crystal structure of TMC-95A bound to the proteasome is now reporting structure-activity relationship data on proteasome inhibition by a TMC-95A analog. The study shows that a simplified version of TMC-95A still retains much of its inhibitory activity. It was carried out by grad student Markus Kaiser, postdocs Michael Groll and Christian Renner, chemist Luis Moroder, and Nobel Prize-winning crystallographer Robert Huber at Max Planck Institute for Biochemistry, Martinsried [*Angew. Chem. Int. Ed.*, **41**, 780 (2002)].

The Max Planck group's paper "invites the synthesis of smaller compounds,"

Danishefsky says. Most researchers in the field believe TMC-95's activity lies in the tripeptide side of the molecule, he says, but an asymmetric center there necessitates a difficult separation that "renders the synthesis impractical." He and Lin hope to create simplified TMC-95 analogs that dispense with the problematic asymmetric center and substantially simplify the biaryl scaffold. "If the biological activity holds up, we can be looking at something quite exciting," Danishefsky says.

Chemistry professor Robert M. Williams and graduate student Brian Albrecht of Colorado State University are close to finishing a synthesis of TMC-95A and B, using what Williams describes as "a different and more concise approach" than the Danishefsky group's synthesis. "We were also the first to report a Stille-type coupling reaction to make the biaryl moiety," Williams notes. Other approaches to TMC-95 synthesis have been pursued by the groups of chemistry professor Masahiro Hirama of Tohoku University, Sendai, Japan, and research professor of chemistry Dawei Ma of the Chinese Academy of Sciences, Shanghai. Hirama's coworker, Tohoku assistant professor of chemistry Masayuki Inoue, notes that the group earlier developed a cis-enamide addition strategy that, like Danishefsky's, is "considered mild enough to be applied to total synthesis" [*Org. Lett.*, **3**, 2863 (2001)].

Chemistry professor Madeleine M. Joullié of the University of Pennsylvania calls the Danishefsky group's total synthesis "elegant and useful." In addition to playing a major role in cellular processes, she says, "the proteasome is a promising target for drug development. Screening of inhibitor intermediates and analogs may produce simpler compounds having the same or better inhibition activity. Therefore, I think the work has broad implications."—STU BORMAN

TMC-95 SYNTHESES
Enamide step ...
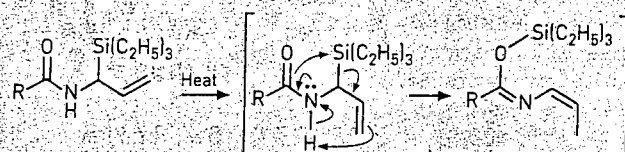
... led to targets
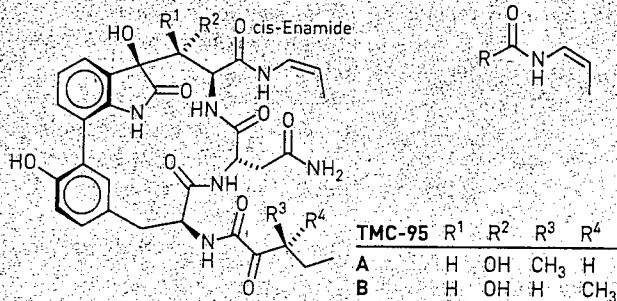
TMC-95	R¹	R²	R³	R⁴
A	H	OH	CH₃	H
B	H	OH	H	CH₃

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MCMR-RMI-S   (70-1y)   8 Jan 2003

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